CREASING STUDIES IN CITRUS

BY

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DECLARATION

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ABSTRACT

Creasing, also known as albedo breakdown, is a preharvest disorder that affects the albedo of citrus fruit causing creases on the surface of the fruit. It is a recurrent problem in Navel and Valencia oranges and can cause individual orchard losses which often exceed 50%. Although the contributing factors are known, the physiological basis of creasing development is unresolved and the current control measures do not prevent creasing satisfactory. Hence, better control measures and further understanding of the physiology of creasing development is required.

The objective of this two-year study was to determine if the position of fruit in a tree, light and carbohydrate manipulation techniques, and albedo mineral nutrients influence creasing development. Furthermore, the most effective application timing of gibberellic acid (GA_3) with the least negative effect on fruit rind colour development and the effectiveness of cytokinins, other products and different root biostimulants to reduce creasing incidence were evaluated.

The position of fruit in the tree and light influenced the development of creasing and the distribution of mineral nutrients in the albedo. Creasing incidence was higher on the south side than on the north side of the tree and fruit from the inside sub-sectors had a greater creasing incidence compared to fruit from the outside sub-sectors. The shady part of outside fruit was more creased compared to the sunny part of the fruit and covering fruit with brown paper bags increased creasing severity. The light manipulation techniques used on the leaves and fruit increased the nitrogen (N), phosphorus (P), potassium (K) and manganese (Mn) concentrations in the albedo and differences in the albedo mineral nutrients amongst the sub-sectors evaluated were observed, but creasing severity or creasing incidence was not significantly correlated with the albedo mineral concentrations at harvest. Albedo mineral concentrations earlier in the season may play a role in creasing development, as creasing severity was significantly correlated with copper

(Cu), K, and Mn concentrations in the albedo during stage II of fruit development. Creasing incidence and albedo mineral concentrations were not affected by any of the carbohydrate manipulation techniques used in this study.

The incidence and severity of creasing was significantly reduced, with a minor negative effect on fruit rind colour development, by the application of $GA_{3,}$ from mid November to mid January. Localised fruit application of CPPU [N-(2-chloro-4-pyridyl)-N-phenylurea], MaxCel (6-Benzyladenine) and CPPU in combination with calcium after physiological fruit drop reduced the incidence and severity of creasing, although creasing incidence was not significantly different from the control. The application of Messenger®, AVG (aminoethoxyvinylglycine) and different root biostimulants did not reduce creasing incidence. The results showed that cytokinins could reduce creasing incidence and justify further studies on application and uptake efficiency. The use of different root biostimulants are not recommended, but it is suggested that treatment effects may be more pronounced over a longer period.

OPSOMMING

Kraakskil is ‘n vooroes abnormalitiet wat die albedo van sitrusvrugte affekteer, deur krake op die oppervlak van vrugte te veroorsaak. Dit is ‘n algemene probleem in Navel en Valencia lemoene en kan boordverliese van tot 50% of soms hoër veroorsaak. Alhoewel die bydraende faktore bekend is, is die fisiologiese basis van kraakskil ontwikkeling onopgelos en die beskikbare beheermaatreëls is nie bevredigend nie. Dus, beter beheermaatreëls en ‘n beter begrip van die fisiologie van kraakskil ontwikkeling is nodig.

Die doel van die twee-jaar studie was om te bepaal of die posisie van vrugte in ‘n boom, lig en koolhidraat manipulasie tegnieke en minerale elemente in die albedo, kraakskil ontwikkeling beïnvloed. Die mees effektiewe toedieningstyd van gibberelliensuur (GA_3) sonder ‘n negatiewe effek op vrugkleur is bepaal en die effektiwiteit van sitokiniene, ander produkte en verskillende wortel biostimulante om kraakskil voorkoms te verminder, is geëvalueer.

Die posisie van vrugte in ‘n boom en lig het kraakskil ontwikkeling en die verspreiding van minerale element in die albedo beïnvloed. Kraakskil voorkoms was hoër aan die suidekant van die boom as aan die noordekant en vrugte in die binnekant van die boom het ‘n groter kraakskil voorkoms as vrugte in die buitekant van die boom gehad. Die skadukant van buitevrugte het meer kraakskil gehad as die sonkant en die toemaak van vrugte met ‘n bruin papiersak het die graad van kraaksil verhoog. Die lig manipulasie tegnieke wat op die blare en vrugte gebruik is, het die stikstof (N), fosfaat (P), kalium (K) en mangaan (Mn) konsentasies in die albedo verhoog en verskille in die albedo minerale elemente tussen sub-sektore is waargeneem, maar betekenisvolle korrelasies is nie tussen die graad en voorkoms van kraakskil en die albedo minerale element konsentrasies by oestyd waargeneem nie. Albedo minerale element konsentrasies vroeër in die seisoen mag ‘n rol speel by kraakskil ontwikkeling, omdat die graad van kraakskil betekenisvol

gekorreleer was met albedo koper (Cu), K, en Mn konsentrasies tydens fase II van vrugontwikkeling. Kraakskil voorkoms en albedo minerale element konsentrasies is nie deur enige van die koolhidraat manipulasie tegnieke geaffekteer nie.

Die voorkoms en graad van kraakskil is betekenisvol verlaag, met ‘n geringe negatiewe effek op vrugkleur, deur die toediening van GA_3 vanaf mid November tot mid Januarie. Gelokaliseerde vrugtoedienings van CPPU [N-(2-chloro-4-piridiel)-N-phenielureum], MaxCel (6-Bensieladenien) en CPPU saam met kalsium na fisiologiese vrugval het die voorkoms en graad van kraakskil verlaag, alhoewel kraakskil voorkoms nie betekenisvol van die kontrole verskil het nie. Die toediening van Messenger®, AVG (amino etoksievinielglisien) en veskillende wortel biostimulante het nie kraakskil voorkoms verlaag nie. Die resultate het getoon dat sitokiniene kraakskil voorkoms kan verlaag en verdere studies op die toediening en opname effektiwiteit word aanbeveel. Die gebruik van verskillende wortel biostimulante word nie aanbeveel nie, maar die effek behoort meer sigbaar te wees na ‘n langer periode van behandeling.

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TABLE OF CONTENTS

This thesis was written according to the language and style required by the journals of the *American Society for Horticultural Science*. Each chapter represents an individual paper and some repetition between the chapters may occur.

1. INTRODUCTION

Fresh citrus fruit is an important component of South Africa's agricultural exports, contributing on average 11% of the agricultural export earnings nationally during the 2002-2004 period (Vermeulen et al., 2006). Citrus fruit quality standards have been established in countries exporting fresh citrus to act as determinants for acceptability in different markets. Fresh citrus fruit aimed for export should be of uniform size with minimum fruit diameter, fruit rind colour, juice content, soluble solids content, acid content and sugar to acid ratio. In addition, fruit must be free from decay, blemishes, pests, diseases and physiological disorders such as granulation, oleocellosis, sunburn, creasing, rind pitting and rind breakdown (Anonymous , 2009). In order to ensure continued access to export markets and remain competitive, strict compliance to these quality standards is of vital importance to South African exporters of fresh citrus fruit (Vermeulen et al., 2006).

Creasing is a recurrent problem in Navels, (Ali et al., 2000; Gilfillan et al., 1980, 1981; Storey et al., 2002) Valencias, (Jona et al., 1989; Jones et al., 1967; Storey et al., 2002) and also Mandarins (Bar-Akiva, 1975). Creasing was first reported in 1938 in South Africa (Le Roux and Crous, 1938). In the past two decades, overall losses of 15 % and significant individual orchard losses which often exceed 50 % have been reported in South Africa (Gilfillan et al., 1981). Moreover, it is a problem worldwide in major citrus producing countries (Alva et al., 2006; Bower, 2000; Jones et al., 1967; Treeby and Storey, 2002) and it is one of the major rind disorders that render large percentages of crops unsuitable for the fresh market (Treeby and Storey, 2002).

Researchers have investigated the physiological basis of the development of creasing and have identified contributing factors in order to develop control measures that will reduce financial losses due to creasing incidence. Application of calcium sprays and gibberellic acid (GA_3) are used commercially to achieve significant reductions in creasing incidence. However, these current control measures do not control creasing satisfactorarily and the physiological basis of creasing development

is still unresolved. Hence, the investigation of better control measures and a further understanding of the physiology of creasing development are required.

2. LITERATURE REVIEW: STUDIES IN CITRUS CREASING

The literature review consists of a description of the physiology of creasing development, the physiological basis of creasing development, the factors that aggravate the incidence of creasing and then lastly the physiological control of creasing specifically looking at calcium and GA_3 spray applications.

2.1 Physiology of creasing

Creasing, also known as albedo breakdown, is a physiological disorder that affects the albedo (white part) of citrus fruit. The physiological basis of creasing development is unknown, but several hypotheses have been proposed. Visual symptoms are observed as creases on the surface of the fruit (Fig. 1).

Fig. 1. Creasing symptoms on 'Washington' navel orange fruit.

2.2 Albedo physiology

The albedo, also known as the mesocarp, is part of the outer layer of a citrus fruit called the rind. It is usually colourless or sometimes tinted and is surrounded by the flavedo, the outer coloured portion of the rind. The thickness and consistence of the albedo varies with the species. It is usually 1-2 mm

thick in some fruit and it merges undetectably into the flavedo towards the outside (Grierson, 2006). Albedo development was categorized into three distinct stages for 'Valencia' oranges by Bain (1958), referred to as stage I, II and III (Storey and Treeby, 1994). During the first two to three months after flower development, the albedo tissue undergoes cell division (stage I), after which cell division ceases and growth in the albedo tissue is limited to cell enlargement and differentiation which continues for six months (stage II). From the end of stage II until harvest time, is the last stage of growth, and is called the maturation stage (stage III) (Storey and Treeby, 1994).

During early fruit development, the albedo tissue consists of a loose network of thin-walled parenchyma cells with numerous large air spaces as part of the inner mesocarp (Grierson, 2006; Jones et al., 1967). As the fruit enlarges, these cells stretch resulting in the development of bulges on the cell walls. These bulges become cylindrical arms as they continue to elongate, and thus the albedo cells become deeply lobed (Grierson, 2006; Jona et al., 1989; Storey and Treeby, 1994) forming an intricate network with large intercellular spaces (Jones et al., 1967). The albedo tissue is composed of cellulose, soluble carbohydrates, flavanoids, amino acids, proteins and pectic substances (Nagy et al., 1985).

2.3 Morphological symptoms of creasing

Creasing is normally detectable at maturity (Gambetta et al., 2000; Jona et al., 1989; Monselise et al., 1976) or post colour break (Storey et al., 2002) and tends to increase as fruit matures (du Plessis and Maritz, 2004; Embleton et al., 1973; Nagy et al., 1982). However, Abadalla et al. (1984) reported that as early as the end of the flowering phase, the anatomic initiation of creasing could be recognised.

Visual symptoms of creasing development are manifested as separations of cells at the middle lamella of the albedo tissue (Treeby et al., 2000), resulting in fractures in the albedo and collapse of the flavedo showing creases on the surface of the fruit (Storey and Treeby, 1994; Treeby et al., 1995). However, for creasing to develop many cell separations should arise in the albedo tissue (Storey and Treeby, 1994).

Creasing is associated with increased polygalacturonase (PG) activity resulting in a higher content of water soluble pectin in the creased fruit (Jona et al., 1989; Monselise et al., 1976). Incorporation of amino acids into proteins is usually higher in creased tissues compared to non-creased tissues (Monselise et al., 1976). Imbalances in hormone levels (especially GA_3) are also associated with creased fruit (Jones et al., 1967; Monselise, 1973). A decrease in cell wall pectin (Jona, 1983), hemicellulose and cell wall polysaccharides is observed in the rind of creased fruit compared to non-creased fruit (Jona et al., 1989).

2.4 Hypothesis for creasing development

The morphological changes in the albedo tissue and the factors that contribute to creasing development do not provide a theoretical basis for its development. Therefore, different hypotheses have been proposed. Holtzhausen (1981) suggested that all the factors which limit the enlargement of the outer layers of the fruit while enlargement of the inner layers is favoured, will result in creasing development. The radial temperature gradient across the fruit and hence a differential water stress across the fruit is also thought to trigger the incidence of creasing (Jones et al., 1967). Storey and Treeby (1994) suggested that creasing develops because there is mechanical stress in the rind which weakens the cohesion between albedo cells at the middle lamella.

Weakening of the outer layers of the fruit is thought to be associated with the early onset of senescence or expression of rapidly progressing senescence (Monselise, 1973; Monselise et al., 1976). In addition, low levels of molybdenum (Mo), sulphur (S) and zinc (Zn) are detrimental in the formation of pectic fractions in the albedo tissue (Bower, 2004) and calcium (Ca) is involved in the bonding of the pectin chains (Bower 2004; Treeby et al., 2000).

3. Factors affecting creasing incidence

3.1 Rind thickness

In general, creasing is limited to fruit with a thinner rind (Holtzhausen, 1981). Jones et al. (1967) suggested that factors such as climate, rootstock, nutrition, and cultural practices that tend to produce fruit with thick rinds tend to reduce the incidence of creasing, and conversely, those that produce a thin rind have the opposite effect. A significant negative correlation between creasing development and rind thickness was observed in California (Ali et al., 2000). However, in South Africa the relationship between rind thickness and the incidence of creasing could not be established (du Plessis and Maritz, 2004).

3.2 Crop load

A low crop load results in a greater proportion of bigger fruit compared to smaller fruit while a larger crop load results in a greater proportion of smaller fruit than bigger fruit (Monselise et al., 1981). Hence, a larger crop load is normally more prone to the incidence of creasing (Jones et al., 1967; Le Roux and Crous, 1938). However, Treeby et al. (1995) reported that creasing incidence is not positively correlated to crop load and Gambetta et al. (2000) did not find any relationship between crop load and creasing incidence.

3.3 Tree heredity

The genetic make-up of a tree has an influence on the severity of creasing (Jones et al., 1967). Generally, early and mid-season selections have significantly higher creasing incidence than the mid-late and late season selections (Treeby et al., 2000). In South Africa, it was observed that ‘Bahianinha’ navels have the lowest incidence of creasing and ‘McClean’ navels have the highest creasing incidence (du Plessis and Maritz, 2004).

3.4 Fruit size

Generally, creasing incidence is more pronounced on smaller fruit with thinner rinds than larger fruit with thicker rinds (du Plessis and Maritz, 2004; Jones et al., 1967; Le Roux and Crous, 1938). On the contrary, Treeby et al. (1995) reported that large and very large fruit had higher proportions of creased fruit compared to smaller fruit.

3.5 Position of fruit on the tree

The temperature gradient across the fruit (Jones et al., 1967), light (Fourie and Joubert, 1957; Holtzhausen, 1981; Jones et al., 1967; Le Roux and Crous, 1938) and mineral nutrients (Bower 2004; Treeby et al., 2000) have an influence on the initial development of creasing. Moreover, allocation of nutrients in citrus fruit depends on the position of a fruit on a tree (Kruger et al., 2005). As a result, creasing is usually greater in the shady part (inside) of the fruit compared to the sunny part (outside) of the fruit (Fourie and Joubert, 1957; Gambetta et al., 2000; Holtzhausen, 1981; Jones et al., 1967; Le Roux and Crous, 1938).

3.5.1 South/north

Jones et al. (1967) reported that creasing severity was higher on the south side of the tree than on the north side of the tree in the northern hemisphere (NH). In the southern hemisphere (SH), Gilfillan et al. (1981) observed a higher creasing incidence on the south side of the tree canopy compared to the north side of the tree.

3.5.2 Inside/outside

In South Africa (SH) it was observed that fruit from the inside sub-sectors of the tree canopy have a significantly greater incidence of creasing than the fruit from the outside sub-sectors of the tree canopy (Verreynne, 2006b).

3.6 Climatic factors

According to Fourie and Joubert (1957) and Holtzhausen (1981), the variation in the incidence and severity of creasing from year to year is attributed to climatic differences. Jones et al. (1967) reported that the temperature range and not temperature *per se* during the early stages of fruit development when cell division and cell enlargement predominates and later when only cell enlargement occurs play a role in creasing development. However, Gambetta et al. (2000) could not establish a relationship between temperature and the incidence of creasing in the periods reported by Jones et al. (1967) in a study on 'Washington' navel oranges (SH). However, high mean relative humidity from full bloom until physiological fruit drop was related to a higher incidence of creasing (Gambetta et al., 2000). On the other hand, a positive correlation between creasing and the average maximum and minimum temperature range in February, prior to flowering was observed (NH) (Ali et al., 2000).Generally, temperature regimes during fruit growth and development play a dominant role in influencing fruit morphology (Rouse and Zerki, 2006).

3.7 Irrigation management

Micro irrigated trees are more sensitive to creasing incidence when compared to drip irrigated trees (du Plessis and Maritz, 2004). Partial root zone drying (PRD) and regulated deficit irrigation (RDI) has been evaluated in relation to creasing incidence (Gonzalez-Altozano and Castel 1999; Treeby et al., 2000; Treeby et al., 2007). Treeby et al. (2007) reported that both RDI and PRD where half of the water volume was applied on both rows and where the half of the water volume was alternatively applied at each irrigation schedule respectively, from June year 1 to July year 3, was associated with lower creasing incidence at harvest (SH). Treeby et al. (2000) also observed a significant reduction in creasing incidence with PRD where rows were watered on alternative irrigation events from spring until harvest time (SH). However, PRD and RDI have a negative effect on fruit size (Gonzalez-Altozano and Castel 1999; Treeby et al., 2007). Holtzhausen (1981) and Le Roux and Crous (1938) could not make a definite conclusion on the effect of irrigation on creasing incidence from their studies.

3.8 Nutritional factors

Albedo mineral nutrition is important in creasing development either through its involvement structurally in the complex formation of pectin or through its influence on the pectin enzyme activity (Bower, 2000). Pectin is primarily composed of homogalacturonan (HGA) and rhamnogalacturonan I (RG I) and is thought to regulate cell-to-cell adhesion at the middle lamella (Carpita and McCann, 2000). Many minerals such as molybdenum (Mo), boron (B), calcium (Ca), zinc (Zn), sulphur (S) and magnesium (Mg) are thought to be involved in the pectin metabolism (Verreynne, 2006a).

It is suggested that Mo acts as a co-factor in ureide synthesis required in the formation of galacturonic acid, a major component of pectin while S is believed to be a component of uronic acid oxidase (Bower, 2004). Boron is in involved in processes such as protein synthesis, transport of sugars, carbohydrate metabolism (Hansch and Mendel, 2009). Moreover, B is thought to cross-link molecules of cell wall polysaccharides called rhamnogalacturonan II (RG II) and thus provides physical strength of the cell wall and is also associated with pectin formation (Hansch and Mendel, 2009; Epstein and Bloom, 2005). Calcium cross-link pectic chains and as well as affect the mechanical properties of the pectic gels (Epstein and Bloom, 2005). Hence, the concentration of Ca in the rind of creased fruit is usually lower than the Ca concentration in the rind of non-creased fruit (Gambetta et al., 2000; Jones et al., 1967; Nagy et al., 1982; Storey and Treeby, 2002; Storey et al., 2002).

Moreover, pectin is influenced by Zn since many Zn-dependant enzymes are involved in carbohydrate metabolism while Mg is involved in carbohydrate metabolism and the synthesis of nucleic acids (Marschner, 1995). Accordingly, lower Mg concentrations in the rind of creased fruit compared to the non-creased fruit were observed at the end of the season (Jones et al., 1967; Storey et al., 2002). In addition, mineral nutrients such as nitrogen (N), phosphorus (P) and potassium (K) have been reported to affect the rind thickness of fruit, and hence influence the development of creasing incidence.

3.8.1 Nitrogen (N)

Nitrogen has more influence on tree growth, tree appearance, fruit production, and fruit quality than any other element (Obreza et al., 2008) and essential N compounds are required for normal cell division, growth and respiration (Obreza et al., 2003). Low levels of N in the soil are associated with fruit with a smooth and thin rind and severe creasing incidence (Le Roux and Crous, 1938). However, the concentration of N in the rind of creased fruit is normally higher compared to non-creased fruit at harvest (Ali et al., 2000; Jones et al., 1967).

3.8.2 Phosphorus (P)

Phosphorus is important for cell division and enlargement, thus plant growth is reduced when the supply of P is too low (Obreza et al., 2003). Haas (1950) observed that low P concentration in the rind was associated with fruit with thick and coarse rinds, whereas high P concentration in the rind resulted in fruit with thinner rinds and more creasing. In addition, the rind of creased fruit were observed to have higher concentrations of P in the rind compared to the rind of non-creased fruit at harvest time (Gambetta et al., 2000).

3.8.3 Potassium (K)

Potassium is important in fruit formation and enhances fruit size, flavour and fruit rind colour (Obreza et al., 2003). Potassium plays a major role in enzyme activation, protein synthesis, stomatal function, tugor related processes and transport of metabolites (Alva et al., 2006). High K levels in the soil results in larger fruit with a thick and coarse rind (Alva et al., 2006; Embleton et al., 1973; Jones et al., 1967) and low levels of K in the soil are associated with fruit with a thinner rind rendering fruit susceptible to creasing (Embleton et al., 1973; Jones et al., 1967; Obreza et al., 2008). Additionally, low concentrations of K in the leaves are associated with the occurrence of creasing and splitting. On the contrary, creased fruit have a higher concentration of K in the rind compared to non-creased fruit (Gambetta et al., 2000; Jones et al., 1967; Storey et al., 2002).

3.9 Rootstock effects

In general, rootstock exerts strong effects on tree vigour, crop load, fruit size and internal fruit quality (Stafford, 1972). The effect of rootstock on creasing incidence may be an indirect effect on fruit size (Treeby et al., 1995) and tree health (Treeby et al., 2000). In South Africa, it was observed that creasing incidence is usually higher on trees on less vigorous rootstocks such as Carrizo citrange and Swingle citrumelo than on trees on more vigorous rootstocks such as Volckameriana and Rough lemon (du Plessis and Maritz, 2004). Contrary to this, in Australia vigorous rootstocks such Rough lemon and Rangpur lime have a higher creasing incidence than less vigorous rootstock such as sweet orange and Cleopatra mandarin (Treeby et al., 1995; Treeby et al., 2000). Thus, Treeby et al. (1995) ranked rootstocks from the lowest to the highest, according to the severity of creasing incidence as follows: sweet orange < Cleopatra mandarin < trifoliate orange < Carrizo Citrange = Troyer Citrange < Rough lemon < Rangpur lime.

3.10 Soil condition

Very little has been reported on the effect of soil condition in relation to creasing incidence but, good soil condition for healthy root growth and development for effective mineral uptake from the soil is important, since mineral nutrients have an influence on the initial development of creasing. In addition, it is possible that improving the root activity would not be only favourable for the uptake of nutrients and water, but also for the synthesis of cytokinins, since root tips are a site for cytokinin synthesis (Salisbury and Ross, 1992; Van Staden and Cook, 1986). This to some extent could improve cell division in the fruit and thereby have an influence on the development of creasing. Hence, soils with *Phytophtora* root rot problems and excessive nematode populations should be treated while heavy and saline soils should be avoided. Nematode control results in more favourable assimilate partitioning in the soil and improved root health and root activity (Miller and Hofman, 1988).

3.11 Time of picking

Creasing becomes more apparent as fruit matures (Nagy et al., 1982) and increases the longer fruit hang on the tree after the normal picking period (du Plessis and Maritz, 2004). Even when no creasing incidence is visible at harvest, creasing can develop within three weeks and reach very high levels (du Plessis and Maritz, 2004).

4. Production strategies to control creasing incidence

4.1 Selection of rootstocks

To reduce the incidence of creasing development it is recommended to select a scion/rootstock combination that is not sensitive to creasing development for new plantings (du Plessis and Maritz, 2004; Treeby et al., 1995). Vigorous rootstocks such as Volckameriana and Rough lemon are recommended under South African conditions (SH) (du Plessis and Maritz, 2004) while less vigorous rootstock such as sweet orange and Cleopatra mandarin are recommended in Australian conditions (SH) (Treeby et al., 1995; Treeby et al., 2000).

4.2 Nutritional remedies

A well-balanced fertilizer programme is important in the production of citrus fruit (Fourie and Joubert, 1957) and a lack of soil fertility is considered to be an important cause of creasing development (Le Roux and Crous, 1938). Summer application of cattle manure plus ammonium sulphate gave less creasing incidence in South Africa (Le Roux and Crous, 1938). On the other hand, application of ammonium phosphate did not reduce creasing incidence in Uruguay (Gambetta et al., 2000) and application of urea phosphate did not reduce creasing incidence when sprayed alone or in combination with GA_3 in South Africa (Gilfillan et al., 1981). However, high phosphate application could reduce rind coarseness and increase creasing incidence (Fourie and Joubert, 1957; Jones et al., 1967).

Application of K fertilizers decrease creasing incidence (Jones et al., 1967). Fourie and Joubert (1957) also observed an increase in creasing incidence and poor storage potential of fruit, where low K rates were applied. Foliar application of Bonus-NPK, a fully soluble, crystalline formulation of potassium nitrate in mid June reduced the incidence of creasing by 20% in 'Nova' tangerine (NH) (Achilea et al., 2002).

Jones et al. (1967) observed that split applications of N resulted in less creasing incidence than did single applications of N in February (NH). An increase in leaf N from 2.54 to 2.71 % by soil application of urea from January to March (NH) slightly reduced creasing incidence and fruit size and resulted in greener fruit at harvest (Embleton et al., 1973). However, it should be noted that a high N fertilization accompanied by an increase in yield and smaller fruit with thin rinds can result in severe creasing (Jones et al., 1967; Le Roux and Crous, 1938). There is a significant interaction between K and N in their effect on fruit size (Miller and Hofman, 1988). Hence, for maximum fruit size an N/K ratio of between 1.6 to 2.2 with N higher than 1.8 % and K higher than 0.9% was recommended (Miller and Hofman, 1988).

4.2.1 Calcium sprays

Creasing develops as a result of cell separations at the middle lamella of the albedo tissue (Treeby et al., 2000) and it is known that Ca cross-links pectic chains as well as affecting the mechanical properties of the pectic gels (Epstein and Bloom, 2005). Thus, the effectiveness of calcium as a control measure for creasing has been investigated in a series of experiments in Australia (Storey et al., 2002; Treeby et al., 2000; Treeby and Storey, 2002) and in South Africa (Verreynne and Phiri, 2008).

4.2.1.1 Role of calcium in fruit development

Ca is the second most abundant mineral nutrient in the rind of citrus fruit (Nagy et al., 1985). A high proportion of the total Ca in the plant tissue is located in the cell walls (Marschner, 1995). Intracellular Ca is also found in the endoplasmic reticulum (ER) and chloroplast and most of the

water-soluble Ca is in the vacuole (Hirschi, 2004). Calcium is required for various structural roles in the cell wall and membranes, as a counter-cation for inorganic and organic anions in the vacuole and as a messenger in the cytosol (White and Broadley, 2003).

Calcium bound as pectate in the middle lamella is essential for strengthening of the cell wall and providing cell wall rigidity by cross linking the pectic chains of the middle lamella (Epstein and Bloom, 2005; Marshner, 1995), as well as affecting the mechanical properties of the pectic gels (Epstein and Bloom, 2005). Calcium is also known to function as a co-factors in enzymatic transformations, act as osmotic regulators and second messengers in metabolic reactions (Marschner, 1995; Nagy et al., 1985; Taiz and Zeiger, 2002). In Ca deficient tissues the activity of polygalacturonase increases resulting in the disintegration of cell walls and collapse of the affected tissues (Marschner, 1995).

Calcium deficiency disorders arise when sufficient Ca is momentarily unavailable to developing tissues. As a result, growing tissues tends to rely on immediate supply of Ca in the xylem which is dependent on transpiration (White and Broadley, 2003). Hence, Ca deficiencies characteristically appear in the merismatic region where cell division is occurring and new cells are being laid down (Hirschi, 2004; Hopkins and Huner, 2004).

4.2.1.2 Uptake and transport of Ca in plants

Calcium is taken up as the divalent cation (Ca^{2+}) and is abundant in most soils and is seldom deficient under natural conditions (Hopkins and Huner, 2004). It is a phloem-immobile nutrient and is transported through plants via the xylem (White and Broadley, 2003). Ca in the xylem sap is translocated upward in the transpiration stream (Hirschi, 2004). Uptake of Ca into the xylem is restricted to regions in which the casparian band between endodermal cells is absent or disrupted or the endodernmal cells surrounding the stele are unsuberized. These regions include the root tips and regions where lateral buds are initiated (White and Broadley, 2003).

Calcium cannot be mobilised from older tissues and redistributed via the phloem, therefore Ca uptake in growing tissues is via the xylem (White and Broadley, 2003). In citrus fruit more Ca moves into the albedo tissue during stage 1 of fruit growth but 100 days after flowering, Ca is then distributed equally between the albedo and the pulp (Storey and Treeby, 2002). Storey and Treeby (2002) suggested that the influx of Ca into oranges may be linked to an increase in transpiration driven xylem flow because at this stage the surface of the fruit is covered in large numbers of stomata resulting in high transpiration rates. Ca uptake by fruit may be influenced by the rootstock, fruit or tree microclimate (Storey et al., 2002) and differences in temperature, relative humidity and rainfall (Peryea, 1994).

4.2.1.3 Efficacy of soil versus foliar applied Ca

Calcium deficiencies normally develop at very low soil pH with the application of too much fertiliser, high soil moisture and heat stress (Bramlage, 1994; Hirschi, 2004). Soil should be maintained at a pH of 6.2 to 6.5 (Bramlage, 1994). Soils high in Mg and K also results in Ca deficiencies (Bramlage, 1994; Hirschi, 2004). For the roots to absorb Ca or any other element, adequate water must be present in the soil. Water stress may directly lower fruit Ca since leaves can draw water and Ca from fruit when severely stressed (Bramlage, 1994).

Calcium sprays such as calcium chloride ($CaCl_2$) or calcium nitrate $Ca(NO_3)_2$ are mostly applied to avoid localized Ca deficiencies in fruit and thus to improve fruit quality (Saure, 2004). Calcium differs from other elements by being imported into fleshy fruit only in small amounts, much less than into leaves (Saure, 2004). Maximum uptake for Ca occurs before 45 to 50 days after flowering (DAF) in citrus (Storey and Treeby, 2002). In apples, it was also observed that the highest uptake was before the June drop (40 to 50 DAF) and after June drop the uptake declined rapidly (Schlegel and Schonherr, 2002). This is because later in the season fruit wax build-up on the rind and this reduces the effectiveness of Ca uptake (Peryea, 1994; Saure, 2004). Handgun sprays appear to have greater effect in wetting the tree than do air blast sprays because of the better coverage of the fruit surface

(Peryea, 1994). However, high concentration of $CaCl_2$ sprays can cause leaf damage, seen as browning and death of the leaf margins (Bramlage, 1994)

4.2.1.4 Effect of calcium

The involvement of Ca in creasing development is associated with its role in bonding of the pectin chains (Bower, 2004), the development of long tuberances in the albedo tissues (Storey and Treeby, 1994) and stretching of the rind during fruit development (Storey and Treeby, 2002). Spraying of citrus fruit with 1% or 2% $Ca(NO_3)_2$ or $CaCl_2$ throughout fruit development from late November to early May increased the proportion of unaffected fruit from 30 to 65-80 % (SH) (Treeby and Storey, 2002) and it was also reported that there was no significant difference between the spray treatments. All Ca spray treatments (amino or glucose-chelated Ca or $Ca(NO_3)_2$ applied alone from mid December to end of January effectively reduced creasing incidence in South Africa (SH) (Verreynne and Phiri, 2008).

4.3 GA_3 applications

GA_3 application reduces the incidence and severity of creasing and is used commercially to control creasing in most citrus producing countries such as America (Coggins, 1969; Embleton et al., 1973; Jones et al., 1967), South Africa (Gilfillan et al., 1980, 1981), Israel (Monselise et al., 1976) and South Australia (Bevington, 1973; Tugwell et al., 1996).

4.3.1 Role of GA in fruit development

Gibberellins were first classified as plant hormones in the 1930's. All gibberellins are acidic, and thus named gibberellic acid (GA_3). It is a naturally occurring hormone or growth regulation substance that is found in varying concentrations in all parts of a plant. The endogenous GA's found in citrus fruit are members of the 13-hydroxylation pathway operating in both vegetative and reproductive tissues (Talon et al., 1997).

Gibberellins promote cell growth because of their involvement in increasing the cell wall plasticity and stimulation of cell division (Salisbury and Ross, 1992). Gibberellins also play a major role in regulating the conversion of chloroplasts to chromoplasts and also in the reversion of chromoplasts to chloroplasts (Coggins and Jones, 1977).

4.3.2 Uptake and application of GA_3 in citrus

The uptake of GA_3 in the fruit is rather poor and attached fruit seem to mobilize gibberellins better than detached fruit (Goldschmidt and Eilati, 1970). Enhancement of GA_3 uptake by acidifying the spray mixture suggests that GA_3 is more easily taken up in its lipophilic, non-dissociated form. GA_3 in citrus is taken up more easily when present in a lipid soluble form and when a humid hydrated environment is present (Greenberg and Goldschmidt, 1988). Translocation of GA_3 in citrus fruit takes place by diffusion (Goldschmidt and Eilati, 1970).

The application of GA_3 results in a delay in fruit rind colour development (Coggins, 1969, 1981). No substantial influence on the internal quality has been reported apart from a slight increase in the juice content (Coggins, 1969). GA_3 can persist in the citrus rind over relatively long periods (Goldschmidt and Eilati, 1970). Since GA_3 delays certain aspects of senescence and ageing of the orange rind it can be applied to reduce certain pre-harvest and post-harvest rind disorders that are associated with senescence (Coggins, 1969; Goldschmidt and Eilati, 1970).

4.3.3 Effect of GA_3

The effectiveness of GA_3 in controlling creasing incidence is associated with its role in stimulating cell division (Holtzhausen, 1981) and cell elongation (Salisbury and Ross, 1992) since the potential of the albedo cells to expand and accommodate cell enlargement after cell division has ceased (Holtzhausen, 1981; Storey and Treeby, 1994) in the albedo is thought to be critical in creasing development. Moreover, the albedo cells of GA_3 treated fruit were observed to be small (Jona et al., 1989), compact (Garcia-Luis et al., 1985), robust and with larger intercellular spaces (Goldschmidt, 1983). GA_3 also acts by reducing the pectin methyl esterase activity (Jona et al., 1989) which is

unusually high in fruits affected by creasing. Jona et al. (1989) also postulated that another role of GA_3 may be the stimulation of DNA, RNA and protein synthesis, leading to the formation of enzymes which either inhibit pectin methyl esterase activity or stimulate insoluble pectin formation.

The effectiveness of GA_3 as a control measure for creasing incidence is dependent on the correct concentration (Bevington, 1973), the spray solution pH (Greenberg and Goldschmidt, 1989; Tugwell et al., 1996) and the timing of application.

4.3.4 GA_3 concentrations

The most effective concentrations are 10 $mg \cdot L^{-1}$ and 20 $mg \cdot L^{-1}$ depending on the location, time of application and severity of creasing. Tugwell et al. (1996) observed that a high volume application of 20 $mg \cdot L^{-1}$ GA_3 concentration was effective in controlling creasing incidence under South Australian conditions. Gilfillan et al. (1980, 1981) recommended a concentration of 10 $mg \cdot L^{-1}$ GA_3 concentration on Navels under South African conditions, while Monselise et al. (1976) recommended 20 $mg \cdot L^{-1}$ GA_3 concentration on Valencia's under Israeli conditions.

4.3.5 Effect of pH of spray mix on efficacy of GA_3

The effectiveness of GA_3 can be increased by acidification of the spray mixture to pH 4.0, by high humidity and conditions of slow drying after application (Gambetta et al., 2000; Gilfillan and Cutting, 1992; Greenberg and Goldschmidt, 1988; Greenberg et al., 1992; Tugwell et al., 1996). Acidifying GA_3 at 20 $mg \cdot L^{-1}$ to pH 4 reduced the incidence of creasing to 11.7% from 16 % at pH 7 (Tugwell et al., 1996). However, GA_3 efficacy is reduced when oil is added to the spray solution (Gilfillan and Cutting, 1992).

4.3.6 GA_3 spray timings

The main period when GA_3 is effective in reducing creasing incidence is during stage I and stage II of fruit growth during the active growth of the rind (Monselise, 1973). Earlier applications when fruitlets are 30-40 mm in diameter reduce creasing development (Bevington, 1973; Gilfillan et al., 1981;

Monselise et al., 1976; Tugwell et al., 1996). In South Africa, application of GA_3 between mid December to mid January (SH) on 'Washington' navel orange is more effective than the mid November spray applications (Gilfillan et al., 1981). In Israel, the best results were obtained when spraying Valencia oranges in July (NH) (Monselise et al., 1976). Under South Australian conditions, GA_3 applications in early January (SH) were recommended on Valencia's (Tugwell et al., 1996). Similarly, Gambetta et al. (2000) observed that applications in January (SH) significantly reduced the percentage of creased fruit.

Later applications when fruitlets are larger than 65 mm are effective but have a strong negative effect on fruit rind colour development (Gambetta et al., 2000; Gilfillan et al., 1974; Gilfillan et al., 1980, 1981; Monselise et al., 1976; Monselise, 1979). Gilfillan et al. (1980, 1981) reported that later sprays in February (SH) were effective but fruit rind colour was severely retarded in 'Washington' navel oranges. Similarly, Bevington (1973) observed that GA_3 application in June (SH) resulted in delayed fruit rind colour development on 'Coastal' navels. Gambetta et al. (2000) also observed a marked delayed fruit rind colour development with GA_3 applications on 'Washington' navel oranges in February (SH).

4.3.7 Effects of GA_3 applications on fruit rind colour development

GA_3 causes a substantial delay in the loss of chlorophyll and a reduction in the rate of accumulation of carotenoids (Coggins and Jones, 1977; Goldschmidt and Eilati, 1970). However, whether high levels of gibberellins maintain chlorophyll synthesis at higher rates or interfere with chlorophyll degradation is unknown. In addition, whether gibberellins exert direct biochemical control over carotenoid synthesis or whether the control is morphological is also unknown (Coggins and Jones, 1977).

5. Conclusion

Creasing is a physiological disorder caused by cell separations at the middle lamella resulting in fractures in the albedo tissue and the collapse of the flavedo showing creases on the surface of the fruit. It is a recurrent problem in citrus orchards, especially on Navels and Valencias. Although the

contributing factors are known, the physiological basis of creasing development is still unresolved. Applications of calcium and gibberellic acid (GA_3) are used commercially to achieve significant reductions in creasing incidence. However, these current control measures do not prevent creasing completely. In addition, the use of GA_3 has a negative effect on fruit rind colour development. Hence, the investigation of better control measures and a further understanding of the physiology of creasing development are required.

Therefore, the objective of the studies in citrus creasing were to determine if the position of a fruit on a tree has an effect on the incidence and severity of creasing and if creasing incidence was more pronounced on the inside part (shaded) or on the outside part (sunny) of the fruit. The relationship between creasing severity or creasing incidence and the albedo mineral concentrations throughout the season and at harvest were also investigated. Additionally, light manipulation techniques and carbohydrates allocation manipulations were assessed in order to provide an insight on the role of light levels in the tree canopy and the effect of carbohydrate manipulation techniques in relation to creasing incidence and the albedo mineral concentrations.

Furthermore, the objective of this study was to determine the most effective application timing of gibberellic acid (GA_3) with the least negative effect on fruit rind colour development. Additionally, the effectiveness of cytokinins, other products and different root biostimulants such as humic acid, fulvic acid, coarse compost and chicken manure in reducing the creasing incidence were evaluated.

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3. RELATIONSHIP OF THE BEARING POSITION OF FRUIT ON A TREE AND THE INCIDENCE AND SEVERITY OF CREASING ON NAVEL ORANGES

ABSTRACT. Creasing is a physiological disorder caused by separation of cells at the middle lamella of albedo tissue resulting in fractures in the albedo and the collapse of the flavedo showing creases on the surface of the fruit. 'Palmer' navel orange trees (2007) and 'Washington' navel orange trees (2009) were used to determine if creasing development is influenced by the position of fruit on the tree and if these differences are related to the mineral nutrient distribution in the albedo. In 2007, each of eight single tree replicates were divided into four sectors, *viz*. north, south, east and west and in each sector fruit were sampled from the inside and outside of the top part of the tree canopy as well as the inside and outside of the bottom part of the tree canopy. In 2009, six single tree replicates were used and fruit were sampled from the outside of the tree on each of the four sectors; north, south, east and west. Fruit were collected biweekly from four weeks after petal drop until the physiological fruit drop after which fruit were collected monthly. Creasing incidence and severity was ranked from highest to lowest as follows in 2007: bottom inside (76.6 %) > bottom outside (73.2 %) > top inside (68.1 %) > top outside (60.7 %). The inside (shady) part of the fruit had a greater creasing severity than the outside (sunny) part of the fruit. Creasing incidence was higher on the south side of the tree than on the north side of the tree. The differences observed in the albedo mineral nutrients amongst the sub-sectors evaluated did not show inverse or similar trends as for creasing incidence or creasing severity. Creasing incidence was not related to the albedo mineral concentrations at harvest but there were significant correlations between creasing severity and Cu, K, and Mn concentrations in the albedo in 2009 during stage II of fruit growth. Therefore, the position of fruit on the tree and the albedo mineral nutrient concentrations earlier in the season may influence creasing development.

Introduction

Creasing is a physiological disorder caused by separation of cells at the middle lamella in the albedo tissues (Treeby et al., 2000) and results in fractures in the albedo and collapse of the flavedo, showing creases on the surface of the fruit (Treeby et al., 1995). Factors such as fruit size, nutrition and crop load play a significant role in creasing incidence and development.

Jones et al. (1967) reported higher creasing severity on the south side than on the north side of the tree in the northern hemisphere (NH). In the southern hemisphere (SH), Gilfillan et al. (1981) also observed a higher creasing incidence on the south side of the tree canopy compared to the north side. Accordingly, creasing was observed to be greater in the shady part of the fruit compared to the sunny part of the fruit (Fourie and Joubert, 1957; Gambetta et al., 2000; Holtzhausen, 1981; Jones et al., 1967; Le Roux and Crous, 1938). The fruit from the inside sub-sector of the tree canopy have a significantly greater incidence of creasing than the fruit from the outside sub-sector of the tree canopy (Verreynne, 2006).

Creasing is also associated with a low calcium (Ca) concentration (Gambetta et al., 2000; Jones et al., 1967; Nagy et al., 1982; Storey and Treeby, 2000; Storey et al., 2002; Treeby et al., 2000; Treeby and Storey, 2002) and high concentrations of potassium (K) (Gambetta et al., 2000; Jones et al., 1967; Storey et al., 2002), phosphorus (P) (Gambetta et al., 2000) and nitrogen (N) (Jones et al., 1967) in the rind of whole fruit at harvest. Lower levels of magnesium (Mg) and sodium (Na) was also found in the rind of creased fruit compared to non-creased fruit at the end of the season (Jones et al., 1967; Storey et al., 2002). Based on mineral analysis of fruit at four weeks post petal fall, Bower (2004) reported that Ca concentration was not a good indicator of creasing development and that the elements molybdenum (Mo), sulphur (S), and zinc (Zn) could potentially be involved in creasing development.

In general, macro and micronutrient distribution in the albedo tissue of fruit varies depending on position of fruit within the canopy (Kruger et al., 2005). Mobile elements in the rind tend to be at

higher concentrations in fruit in the inside of the tree canopy compared to fruit in the outside of the tree canopy and immobile elements in the rind occur at higher concentrations in fruit in the outside of the tree canopy compared to fruit in the inside of the tree canopy (Kruger et al., 2005). Storey et al. (2002) reported variations in nutrient concentration in the albedo tissue of non-creased and creased fruit depending on the position of fruit in a tree without reporting on the incidence of creasing in the different positions.

Hence, the aim of the studies were to determine if the position of a fruit on a tree had an effect on the incidence and severity of creasing and if creasing incidence was more pronounced on the inside part (shaded) or on the outside part (sunny) of the fruit. Furthermore, the relationships between creasing severity and the albedo mineral nutrients throughout the season and at harvest was determined.

Materials and Methods.

Plant material and sampling. The studies were conducted in 2007 and 2009, at two different orchards with a history of severe creasing incidence. In the first year (2007) a commercial orchard of 'Palmer' navel orange trees in Addo (33°32'S, 25°45'E) in the Eastern Cape, South Africa was used. Tree spacing was 6m between rows and 4m in rows and the row direction of the orchard was north to south. Each tree replicate (8 replicates) was divided into four sectors, *viz*. north, south, east and west. In each sector, fruit were harvested from four different sub-sectors, from the inside and outside of the top part of the tree canopy as well as the inside and outside of the bottom part of the tree canopy. A total of sixteen positions were used and these sixteen positions were replicated eight times.

In the second year (2009), the study was conducted in a commercial orchard in Citrusdal (32°36'S, 19°1'E) in the Western Cape, South Africa on 'Washington' navel orange trees. Tree spacing was 6m between rows and 6m in rows and the row direction of the orchard was north to south. Six single tree replicates per sampling date were used. To distinguish the shaded and sun exposed part of the fruit, the sun exposed part of the fruit was marked with a pen before removal from the tree. Fruit were

collected biweekly from four weeks after petal drop (28 Nov. 2008) until the physiological fruit drop after which fruit were collected monthly at the following dates: 11 Dec. 2009, 23 Jan. 2009, 26 Feb. 2009, 31 Mar. 2009 and 23 Apr. 2009. Fruit were randomly selected from the outside part of the tree canopy at eye-sight level.

Measurements and data collection. In the first year (Addo), the fruit were sampled at commercial harvest on 5 June 2007. Six fruit were picked from each of the sixteen positions and were evaluated for creasing severity, fruit diameter and albedo mineral concentration. Creasing severity was evaluated on a score of 0 to 4 for each fruit; the orange was divided into four equivalent imaginary spheres. If no sphere was creased it was designated a zero, for a sphere that showed creasing the score was noted from 1 (one sphere) to 4 (all spheres of the fruit). Creasing incidence as a percentage of fruit evaluated was also calculated and in addition the diameter of each fruit was measured using an electronic calliper (CD-6″ C, Mitutoyo Corp, Toyko, Japan). The albedo tissue of the shaded and sun exposed part of each fruit from the outside sub-sectors was removed, oven dried at 60^{O}C and stored in vials for a complete mineral analysis.

At each sampling date the fruit from Citrusdal (2009) were cut into half and the albedo tissue of the shaded and sun exposed part of each fruit was removed, oven dried at 60^{O}C, ground into a powder and stored in vials for a complete mineral analysis. At commercial harvest on 5 May 2009, an on-tree evaluation of creasing severity and fruit rind colour was carried out the same as the previous year (Addo) as descreibed above. Twenty fruit per single tree were evaluated. Five fruit per single tree replicate were evaluated at random from the outside of the tree at eye-sight level on each of the four sectors of the tree; north, south, east and west. Creasing severity and fruit rind colour and the creasing incidence was calculated as a percentage of the fruit evaluated.

Statistical analysis. Analysis of variance was performed using the computer program SAS (Statistical Analysis System) Enterprise Guide 3. Duncan multiple range test at P= 0.05 was used to test the

treatment effects. The correlation between albedo mineral content and creasing incidence or severity was demonstrated with the Pearson's correlation coefficients and only r values ≥ 0.5 were considered physiological significant. All percentages data were arcsine transformed to degrees asin√ before the statistical calculations were made.

Results

2007

Creasing incidence was generally high (>45%) and creasing incidence among all sub-sectors evaluated was ranked from highest to lowest as follows: bottom inside > bottom outside > top inside > top outside (Table 1). On average, fruit from the inside sub-sectors had significantly greater creasing incidence than fruit from the outside sub-sectors. However, there were no significant differences between fruit sampled from the top versus the bottom part of the tree. Creasing incidence amongst the sides of the tree viz. north, south, east, west was ranked from the highest to the lowest as follows: south > west > east > north. Hence, creasing incidence was significantly higher on the south side than on the north side of the tree. There were no significant differences between fruit sampled from the west side and the east side of the tree (Table 1).

[Table 1]

A similar trend as described above for creasing incidence was observed for creasing severity. The exception was that fruit sampled from the west side had a significantly greater creasing severity than fruit sampled from the east side of the tree (Table 1). There were no significant differences in the fruit diameter of fruit sampled from the different sub-sectors (Table 1). Accordingly, very weak non significant correlations between fruit diameter and creasing incidence (r= -0.09) or creasing severity (r= -0.06) were observed (Table 2). However, only P,K, and Cu concentrations in the albedo tissue were significant, although very weak negative correlations with creasing severity were observed (Table 12).

[Table 1 and 2]

Even though significant differences in the albedo macronutrient concentrations (Table 3) and the albedo micronutrient concentrations (Table 4) of fruit sampled from the different sub-sectors were observed, no inverse or similar trends as for creasing incidence or creasing severity (Table 1) with regards to position of fruit in the tree were observed. These results were confirmed by very weak correlations between albedo macronutrient or micronutrient concentrations and creasing incidence (%) or creasing severity (Table 2).

[Table 2, 3 and 4]

Creasing severity was higher in the shady part of fruit compared to the sunny part of the fruit in almost all the sub-sectors evaluated (Table 5). Nitrogen (N) and potassium (K) concentrations in the albedo tissue were significantly greater in the shady part of the fruit than the sunny part of the fruit in only two of the sub-sectors (Table 6), while phosphorus (P) and calcium (Ca) concentrations in the albedo tissue were significantly lower in the shady part of the fruit in only one sub-sector (Table 7), whereas the concentration of magnesium (Mg) in the albedo tissue was significantly greater in the shady part of the fruit in only one sub-sector (Table 8).

[Table 5, 6, 7 and 8]

Sodium (Na) (Table 8), manganese (Mn) (Table 9), Iron (Fe) (Table 9) and copper (Cu) (Table 10) concentrations in the albedo tissue were not significantly different between the shady part of the fruit compared to the sunny part of the fruit in any of the sub-sectors. Zinc (Zn) concentration in the albedo tissue was significantly lower in the shady part of the fruit compared to the sunny part of the fruit in only one sub-sector (Table 10) and boron (B) concentration in the albedo tissue was significantly lower in the shady part of the fruit compared to the sunny part of the fruit in two sub-sectors and higher in the shady part of the fruit compared to the sunny part of the fruit in only one sub-sector (Table 11).

[Table 10 and 11]

The relationship between creasing severity and mineral nutrient concentrations in the albedo tissue in the sunny part of the fruit or the shady part of the fruit sampled at harvest resulted in very weak and non significant correlations (Table 12). However, only B concentration in the albedo tissue in the shady part of the fruit was significant, although very weak negative correlations with creasing severity were observed (Table 12).

[Table 12]

2009

Creasing severity was significantly higher on the shady part of the fruit compared to the sunny part of the fruit (Table 13). N, P, and K concentrations in the albedo tissue were not significantly different between the shady part of the fruit compared to the sunny part of the fruit throughout the season, except in February where the N concentration in the albedo tissue was greater in the shady part of the fruit compared to the sunny part of the fruit (Fig. 1). Ca and Mg concentrations in the albedo tissue were lower in the shady part of the fruit compared to the sunny part of the fruit throughout the season but these differences were only significant in February, March, and April for Ca and in February and March for Mg.

[Table 13 and Fig 1]

There were no significant differences between the albedo mineral concentrations in the shady part of the fruit compared to the sunny part of the fruit for Na, Fe, Cu, Zn and B (Fig. 2). Only Mn concentrations in the albedo tissue were lower in the shady part of the fruit compared to the sunny part of the fruit throughout the season from December, although it was only significant in March (Fig 2).

[Fig 2]

Albedo macronutrient and micronutrient concentrations in both the sunny or the shady part of the fruit sampled at the end of November did not show any strong correlations with creasing severity except for Cu concentration in the albedo tissue in the shady part of the fruit (r=0.91) (Table 14).

[Table 14]

In mid December, only K concentrations in the albedo tissue in the sunny part of the fruit was positively correlated with creasing severity (r=0.88) while only Mn concentrations in the albedo tissue in the sunny part of fruit was negatively correlated with creasing severity (r=-0.93) (Table 15).

[Table 15]

The relationship between creasing severity and macronutrient or micronutrient concentrations in the albedo tissue in both the sunny part of the fruit or the shady part of the fruit sampled at the end January (Table 16), end of February (Table 17), end of March (Table 18) and at the end of April (Table 19) resulted in non-significant correlations.

[Table 16, 17, 18 and 19]

Discussion

It was observed that the position of fruit in the tree play a significant role in creasing development. Creasing incidence was higher on the south side of the tree than on the north side of the tree. This trend was similar to what was observed earlier in South Africa (SH) (Gilfillan et al., 1981). Additionally, and concurring with Verreynne (2006), fruit from the inside sub-sectors had greater creasing incidence than fruit from the outside sub-sectors, with the tendency of the fruit to be more creased on the shady part of the fruit compared to the sunny part of the fruit which was also consistent with previous work (Fourie and Joubert, 1957; Holtzhausen, 1981; Jones et al., 1967; Le Roux and Crous., 1938). Therefore, it is assumed that light levels in the tree canopy could play an important role in creasing development.

Creasing incidence is normally more pronounced on smaller fruit compared to larger fruit (du Plessis and Maritz, 2004; Holtzhausen, 1981; Jones et al., 1976; Le Roux and Crous, 1938). However, in our results, there were no significant differences in fruit diameter from fruit sampled in the different sub-sectors. Hence, the differences in the creasing incidence were not due to fruit size as shown by the very weak correlations between the fruit diameter and creasing incidence.

In citrus fruit, phloem mobile elements in the rind tend to be at higher concentrations in fruit in the inside of the tree canopy compared to the outside and phloem immobile elements occur in the rind at higher concentrations in fruit in the outside compared to fruit in the inside of the tree canopy (Kruger et al., 2005). Our results showed some significant differences in the albedo mineral concentrations amongst the sub-sectors evaluated, but no inverse or similar trends as for creasing incidence or creasing severity were observed. Therefore, it is possible that albedo mineral concentrations at harvest did not play a role in the development of creasing. This was also confirmed by the very weak correlations between the albedo macronutrient or micronutrient concentrations and creasing incidence or creasing severity.

When comparing the albedo mineral concentrations in the inside part of the fruit and the outside part of the fruit at harvest, the inside part of the fruit had a significantly higher N, K and Mg , lower P, Ca and Zn and either lower or higher B in only one or two out of the eight sub-sectors evaluated. As a result, a consistenT difference between albedo mineral concentrations in the shady part and the sunny part of the fruit could not be established. However, it is known that the shady part of the fruit has a higher concentration of phloem mobile elements in the rind than the sunny part of the fruit and that phloem immobile elements occur at a higher concentration in the sunny part of the fruit than the shaded part of the fruit (Kruger et al., 2005). Storey et al. (2002) reported that there is a negative correlation between Ca and a positive correlation between K and creasing incidence. Hence, creased fruit have a lower Ca and higher K in the albedo tissue than non-creased fruit (Storey et al, 2002; Treeby et al., 2000). However, our results showed very weak correlations between creasing severity

and albedo mineral concentrations at harvest in either the sunny part of the fruit or the shady part of the fruit.

During the specific months of stage II and stage III of citrus fruit development, the shady part of the fruit had a greater concentration of N in the albedo than the sunny part of the fruit and Mg, Ca and Mn in the albedo occurred at a greater concentration in the sunny part of the fruit than the shady part of the fruit, but these elements were not significantly correlated with creasing severity. However, during stage II of fruit growth, creasing severity was positively correlated with Cu in the shady part of the fruit and positively correlated with K and Mn in the sunny part of the fruit. In most cases a decrease in Cu is correlated with distinct metabolic changes and inhibition of plant growth (Marschner, 1995).

In conclusion, creasing development and distribution of mineral nutrients in the fruit are influenced by the position of fruit in the tree. Hence, light levels in the tree canopy could play a role in creasing development. Differences in the albedo mineral concentrations at harvest between the shady part and the sunny part of the fruit were not observed, but N, Mg, Ca and Mn differed significantly between the shady part and sunny part of the fruit during specific months of stage II and stage III of fruit growth. As a result, creasing incidence was not related to the albedo mineral concentrations at harvest, but there was a significant relationship between creasing severity and Cu, K, and Mn concentrations in the albedo during stage II of fruit growth. Therefore, albedo mineral nutrients earlier in the season may influence creasing development. Additional research on the role and the relationship between creasing severity and the albedo mineral nutrients earlier in the season is required. Fruit should be sampled from the outside sub-sectors and only the inside part of the fruit should be used for further laboratory analysis. In instances where distinct differences in the creasing incidence amongst the different sub-sectors are observed, selective harvesting may be done to reduce the proportion of creased fruit in the packhouse. Furthermore, pruning to ensure light penetration in the tree may be important in reducing creasing incidence.

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Table 1. Creasing incidence, creasing severity and fruit diameter on different positions (sub-sectors) of 'Palmer' navel trees harvested in 2007 in Addo.

POSITION WITHIN TREE		Diameter	Creasing severity	Creasing incidence
		--mm--	*--0-4--*	*--%--*
North	Top outside	69.9	0.9 d[z]	52.3
	Top inside	72.9	1.4 bcd	64.1
	Bottom outside	67.9	1.4 bcd	64.3
	Bottom inside	69.9	1.1 cd	64.3
South	Top outside	68.7	1.7 abcd	69.3
	Top inside	69.9	1.9 ab	81.0
	Bottom outside	63.6	1.8 abc	78.4
	Bottom inside	68.1	2.3 a	83.3
East	Top outside	71.1	0.9 d	47.4
	Top inside	68.7	1.1 bcd	55.7
	Bottom outside	68.6	1.7 abcd	83.3
	Bottom inside	69.8	1.7 abcd	78.6
West	Top outside	69.6	1.5 abcd	73.9
	Top inside	68.8	1.8 abc	71.6
	Bottom outside	68.8	2.0 ab	66.7
	Bottom inside	70.4	2.3 a	80.1
	P-value	0.1861	0.0019	0.1106
	SE	1.6492	0.2715	9.1072
Average	Top outside	69.8	1.3	60.7
	Top inside	70.1	1.6	68.1
	Bottom outside	67.2	1.7	73.2
	Bottom inside	69.6	1.9	76.6
Source:	Df			
Treatment	15			
North vs. South	1	0.0282	0.0001	0.0109
West vs. East	1	0.7990	0.0078	0.2553
Outside vs. Inside	1	0.0574	0.0050	0.0207
Top vs. Bottom	1	0.1117	0.0992	0.2542

[z]Means in each column with the same letter are not significantly different at the 5% level (Duncan)

Table 2**.** Relationship between creasing incidence (%) or creasing severity and fruit diameter, macronutrient or micronutrient concentrations in the albedo tissue of 'Palmer' navel fruit sampled in 2007 in Addo.

Fruit diameter vs. creasing %, creasing severity			
		r	*P*-value
Fruit diameter	Creasing %	-0.09	0.3538
Fruit diameter	Creasing severity	-0.06	0.5918
Creasing % vs. mineral nutrients in albedo			
Creasing %	N	0.11	0.2716
Creasing %	P	0.05	0.6268
Creasing %	K	0.12	0.1958
Creasing %	Ca	-0.14	0.1322
Creasing %	Mg	0.06	0.5190
Creasing %	Na	-0.02	0.7976
Creasing %	Mn	0.11	0.2675
Creasing %	Fe	0.10	0.3052
Creasing %	Cu	0.01	0.9156
Creasing %	Zn	0.06	0.5096
Creasing %	B	-0.20	0.0360
Creasing severity vs. mineral nutrients in albedo			
Creasing severity	N	-0.06	0.5765
Creasing severity	P	-0.27	0.0068
Creasing severity	K	-0.23	0.0270
Creasing severity	Ca	-0.07	0.5154
Creasing severity	Mg	-0.13	0.2058
Creasing severity	Na	-0.03	0.7353
Creasing severity	Mn	0.15	0.1539
Creasing severity	Fe	0.06	0.5618
Creasing severity	Cu	-0.27	0.0084
Creasing severity	Zn	-0.10	0.3191
Creasing severity	B	-0.18	0.0781

Table 3. Macronutrient concentrations in the albedo tissue of fruit sampled from different positions (sub-sectors) from 'Palmer' navel trees in 2007 in Addo.

POSITION WITHIN TREE		N	P	K	Ca	Mg
		----------%----------				
North	Top outside	0.73 abcde[z]	0.032 bcd	0.28 bcd	0.50 ab	0.056 ab
	Top inside	0.86 a	0.039 a	0.36 a	0.38 d	0.062 a
	Bottom outside	0.76 abcd	0.034 abc	0.33 ab	0.49 abc	0.057 ab
	Bottom inside	0.81 abc	0.034 abc	0.34 ab	0.44 bcd	0.057 ab
South	Top outside	0.65 de	0.031 bcd	0.21 efg	0.54 a	0.046 cd
	Top inside	0.81 abc	0.029 d	0.24 cdefg	0.38 d	0.044 cd
	Bottom outside	0.72 bcde	0.034 abc	0.25 cdef	0.49 abc	0.050 bcd
	Bottom inside	0.83 abc	0.031 bcd	0.30 abcd	0.40 d	0.056 ab
East	Top outside	0.72 bcde	0.032 bcd	0.25 cdef	0.54 a	0.053 bc
	Top inside	0.40 f	0.036 ab	0.31 abc	0.43 dc	0.056 ab
	Bottom outside	0.70 cde	0.035 abc	0.26 cde	0.52 a	0.050 bcd
	Bottom inside	0.85 ab	0.034 abc	0.29 abcd	0.43 bcd	0.053 bc
West	Top outside	0.62 e	0.031 bcd	0.18 g	0.55 a	0.041 d
	Top inside	0.74 abcde	0.030 cd	0.22 efg	0.45 bcd	0.046 cd
	Bottom outside	0.61 e	0.029 d	0.19 fg	0.55 a	0.044 cd
	Bottom inside	0.71 cde	0.030 cd	0.24 defg	0.48 abc	0.048 bcd
	P-value	0.0001	0.0007	0.0001	0.0001	0.0001
	SE	0.0419	0.0017	0.0209	0.02193	0.0030
Average	Top outside	0.68	0.032	0.23	0.53	0.049
	Top inside	0.70	0.033	0.28	0.41	0.052
	Bottom outside	0.70	0.033	0.26	0.52	0.050
	Bottom inside	0.80	0.032	0.29	0.44	0.050
Source:	df					
Treatment	15					
North vs. South	1	0.1653	0.0015	0.0001	0.9354	0.0001
West vs. East	1	1.0000	0.0003	0.0001	0.0961	0.0003
Outside vs. Inside	1	0.0060	0.8749	0.0262	0.6308	0.3048
Top vs. Bottom	1	0.0027	0.6277	0.0001	0.0001	0.0363

[z]Means in each column with the same letter are not significantly different at the 5% level (Duncan)

Table 4. Micronutrient concentrations in the albedo tissue of fruit sampled from different positions (sub-sectors) of 'Palmer' navel trees in 2007 in Addo.

POSITION WITHIN TREE		Na	Mn	Fe	Cu	Zn	B
		---------- *mg·kg^{-1}* ----------					
North	Top outside	141 bcdef[z]	0.79 d	52.4 b	2.29 ab	6.57 abc	24.2 ab
	Top inside	245 a	4.00 ab	11.9 b	1.71 cd	7.29 ab	24.3 ab
	Bottom outside	199 abcd	0.43 d	47.7 b	2.43 a	8.36 a	24.6 ab
	Bottom inside	218 abc	3.86 ab	42.0 b	1.57 cde	6.57 abc	24.0 ab
South	Top outside	113 def	0.07 d	32.5 b	1.79 bcd	5.29 bc	24.4 ab
	Top inside	129 cdef	2.72 c	10.6 b	1.29 de	4.71 c	24.7 c
	Bottom outside	106 def	0.21 d	35.8 b	2.36 a	6.57 abc	24.5 ab
	Bottom inside	169 abcde	3.71 ab	16.0 b	2.00 abc	5.79 bc	23.6 ab
East	Top outside	99 ef	0.57 d	26.5 b	2.00 abc	5.71 bc	24.8 ab
	Top inside	216 abc	4.00 ab	21.3 b	1.71 cd	7.29 ab	23.9 ab
	Bottom outside	146 bcdef	0.50 d	42.6 b	2.07 abc	5.57 bc	25.1 a
	Bottom inside	227 ab	4.29 a	63.9 b	1.29 de	5.71 bc	24.3 ab
West	Top outside	65 f	0.29 d	175.8 a	1.42 de	7.36 ab	23.6 ab
	Top inside	83 ef	3.43 b	9.4 b	1.42 de	5.29 bc	23.0 ab
	Bottom outside	88 ef	2.14 c	38.6 b	1.14 e	5.86 bc	24.2 abc
	Bottom inside	109 def	3.57 ab	15.3 b	1.29 de	5.42 bc	22.7 b
	P-value	0.0001	0.0001	0.0209	0.0001	0.0176	0.0024
	SE	30.182	0.2406	27.781	0.7198	0.6785	0.6428
Average	Top outside	105	0.43	71.8	1.89	6.23	24.2
	Top inside	169	3.43	13.2	1.53	6.15	23.9
	Bottom outside	135	0.82	41.2	1.99	6.59	24.6
	Bottom inside	181	3.86	34.3	1.54	5.87	23.6
Source:	df						
Treatment	15						
North vs. South	1	0.0014	0.0008	0.5000	0.2432	0.0012	0.0334
West vs. East	1	0.0001	0.9166	0.2835	0.0004	0.8528	0.0165
Outside vs. Inside	1	0.1296	0.0038	0.6893	0.4692	0.8956	0.1181

[z]Means in each column with the same letter are not significantly different at the 5% level (Duncan)

Table 5. Differences in creasing severity between the outside (sunny) and inside (shady) part of fruit sampled from different positions from the outside sub-sectors of 'Palmer' navel trees in 2007 in Addo.

POSITION WITHIN TREE		**Creasing severity**		
		Sun	**Shade**	***P*-value**
		--------*0-2*--------		
North	Top outside	0.21	0.65	0.0215[z]
	Bottom outside	0.43	0.88	0.1825
South	Top outside	0.59	1.22	0.0235[z]
	Bottom outside	0.66	1.19	0.0001[z]
East	Top outside	0.13	0.68	0.0049[z]
	Bottom outside	0.36	1.38	0.0292[z]
West	Top outside	0.31	1.33	0.0017[z]
	Bottom outside	0.61	1.13	0.0241[z]

[z]Means for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (T-test)

Table 6. Differences in N and K concentrations in albedo tissue between the outside (sunny) and inside (shady) part of the fruit sampled from different positions from the outside sub-sectors of 'Palmer' navel trees in 2007 in Addo.

		Nitrogen(N)			Potassium (K)		
POSITION WITHIN TREE		**Sun**	**Shade**	***P*-value**	**Sun**	**Shade**	***P*-value**
		--------%--------			--------%--------		
North	Top outside	0.73	0.74	0.6360	0.28	0.28	0.7152
	Bottom outside	0.73	0.78	0.0439[z]	0.33	0.33	0.8641
South	Top outside	0.65	0.65	0.4618	0.09	0.21	0.0141[z]
	Bottom outside	0.70	0.73	0.0668	0.25	0.26	0.2150
East	Top outside	0.71	0.72	0.8353	0.23	0.25	0.0388[z]
	Bottom outside	0.68	0.72	0.1596	0.27	0.25	0.2695
West	Top outside	0.61	0.62	0.5275	0.16	0.19	0.1087
	Bottom outside	0.60	0.62	0.0264[z]	0.08	0.23	0.0583

[z]Means for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (T-test)

Table 7. Differences in P and Ca concentrations in albedo tissue between the outside (sunny) and inside (shady) part of the fruit sampled from different positions from the outside sub-sectors of 'Palmer' navel trees in 2007 in Addo.

		Phosphorus (P)			Calcium (Ca)		
POSITION WITHIN TREE		**Sun**	**Shade**	***P*-value**	**Sun**	**Shade**	***P*-value**
		--------%--------			--------%--------		
North	Top outside	0.031	0.034	0.1723	0.53	0.48	0.0347
	Bottom outside	0.034	0.034	-	0.51	0.47	0.0029
South	Top outside	0.031	0.031	0.1723	0.54	0.54	0.1375
	Bottom outside	0.031	0.037	0.3632	0.50	0.49	0.0172
East	Top outside	0.031	0.030	0.3559	0.55	0.52	0.8049
	Bottom outside	0.036	0.033	0.0300[z]	0.55	0.50	0.2919
West	Top outside	0.030	0.034	0.3739	0.56	0.52	0.2552
	Bottom outside	0.029	0.032	-	0.58	0.47	0.0017[z]

[z]Means for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (T-test)

Table 8. Differences in Na and Mg concentrations in albedo tissue between the outside (sunny) and inside (shady) part of the fruit sampled from different positions from the outside sub-sectors of 'Palmer' navel trees in 2007 in Addo.

		Magnesium (Mg)			Sodium (Na)		
POSITION WITHIN TREE		**Sun**	**Shade**	***P*-value**	**Sun**	**Shade**	***P*-value**
		--------*%*--------			------*mg·kg^{-1}*------		
North	Top outside	0.056	0.054	0.3559	139.43	142.71	0.8592
	Bottom outside	0.057	0.054	0.1723	202.00	197.57	0.7182
South	Top outside	0.044	0.046	0.0082[z]	107.71	118.86	0.4355
	Bottom outside	0.049	0.049	0.6109	106.14	97.43	0.4468
East	Top outside	0.046	0.050	0.3559	93.86	103.57	0.2118
	Bottom outside	0.049	0.048	1.0000	141.14	112.17	0.4117
West	Top outside	0.037	0.044	0.3739	61.86	75.20	0.5533
	Bottom outside	0.041	0.043	0.6109	91.29	93.50	0.2821

[z]Means for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (T-test)

Table 9. Differences in Mn and Fe concentrations in albedo tissue between the outside (sunny) and inside (shady) part of the fruit sampled from different positions from the outside sub-sectors of 'Palmer' navel trees in 2007 in Addo.

		Manganese (Mn)			Iron (Fe)		
POSITION WITHIN TREE		**Sun**	**Shade**	***P*-value**	**Sun**	**Shade**	***P*-value**
		------$mg \cdot kg^{-1}$------			------$mg \cdot kg^{-1}$------		
North	Top outside	0.86	0.71	0.6036	37.43	67.4	0.3260
	Bottom outside	0.43	0.43	1.0000	42.71	40.71	0.8456
South	Top outside	0.00	0.14	0.1723	31.43	33.57	0.5171
	Bottom outside	0.29	0.14	0.3632	28.57	35.71	0.4098
East	Top outside	0.43	0.71	0.3559	27.57	25.14	0.8557
	Bottom outside	0.57	0.16	0.3559	50.14	27.83	0.6004
West	Top outside	0.50	0.20	0.6042	301.29	57.20	0.2445
	Bottom outside	1.29	3.50	0.0580	55.14	9.17	0.1347

[z]Means for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (T-test)

Table 10. Differences in Cu and Zn concentrations in albedo tissue between the outside (sunny) and inside (shady) part of the fruit sampled from different positions from the outside sub-sectors of 'Palmer' navel trees in 2007 in Addo.

		Copper (Cu)			Zinc (Zn)		
POSITION WITHIN TREE		**Sun**	**Shade**	***P*-value**	**Sun**	**Shade**	***P*-value**
		-----$mg \cdot kg^{-1}$-----			-----$mg \cdot kg^{-1}$-----		
North	Top outside	2.14	2.14	1.0000	6.43	6.71	0.4571
	Bottom outside	2.57	2.29	0.1723	10.57	6.14	0.2540
South	Top outside	1.71	1.86	1.0000	5.00	5.57	0.5686
	Bottom outside	2.43	2.29	0.3632	7.00	6.14	1.0000
East	Top outside	2.00	2.00	0.6036	5.86	5.57	0.0300[z]
	Bottom outside	2.00	2.17	0.6891	5.57	5.50	0.5546
West	Top outside	1.29	1.80	0.1778	9.43	5.20	0.1321
	Bottom outside	1.14	1.17	-	6.71	4.67	0.2805

[z]Means for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (T-test)

Table 11. Differences in B concentrations in albedo tissue between the outside (sunny) and inside (shady) part of the fruit sampled from different positions from the outside sub-sectors of 'Palmer' navel trees in 2007 in Addo.

		Boron (B)		
POSITION WITHIN TREE		**Sun**	**Shade**	***P*-value**
		-----*mg·kg*$^{-1}$-----		
North	Top outside	24.86	23.57	0.0781
	Bottom outside	25.43	24.00	0.0465[z]
South	Top outside	24.71	21.14	0.0488[z]
	Bottom outside	24.14	24.86	0.0067[z]
East	Top outside	24.14	25.43	0.2308
	Bottom outside	26.14	23.33	0.3341
West	Top outside	23.71	23.00	0.8149
	Bottom outside	24.14	23.83	0.8220

[z]Means for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (T-test)

Table 12. Relationship between creasing severity and macronutrient or micronutrient concentrations in the albedo tissue of 'Palmer' navel fruit in the outside (sunny) and inside (shady) part of fruit sampled at harvest from different positions from the outside sub-sectors in 2007 in Addo.

Creasing severity vs. mineral nutrients in the albedo in the sunny part of fruit			
		r	*P*-value
Creasing severity (0-2)	N	0.00	0.9902
Creasing severity	P	-0.16	0.2323
Creasing severity	K	0.06	0.6250
Creasing severity	Ca	-0.20	0.1335
Creasing severity	Mg	0.11	0.4191
Creasing severity	Na	0.02	0.8624
Creasing severity	Mn	-0.06	0.6396
Creasing severity	Fe	0.21	0.1214
Creasing severity	Cu	0.06	0.6484
Creasing severity	Zn	0.02	0.8639
Creasing severity	B	-0.37	0.0053
Creasing severity vs. mineral nutrients in the albedo in the shady part of fruit			
Creasing severity (0-2)	N	-0.07	0.6231
Creasing severity	P	-0.01	0.9284
Creasing severity	K	-0.12	0.3912
Creasing severity	Ca	-0.09	0.5476
Creasing severity	Mg	-0.26	0.0658
Creasing severity	Na	-0.09	0.5401
Creasing severity	Mn	-0.04	0.7720
Creasing severity	Fe	-0.16	0.2468
Creasing severity	Cu	-0.10	0.5027
Creasing severity	Zn	0.05	0.7301
Creasing severity	B	-0.19	0.1758

Table 13. Differences in creasing severity between the outside (sunny) and inside (shady) part of fruit sampled from different positions from the outside sub-sectors of 'Washington' navel trees in 2009 in Citrusdal.

	Sun	**Shade**	***P*-value**
Creasing severity (0-2)	0.025	0.308	0.0206[z]

[z]Means for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (T-test)

Table 14. Relationship between creasing severity at harvest and macronutrient or micronutrient concentrations in the albedo tissue of 'Washington' navel fruit in the outside (sunny) and inside (shady) part of fruit sampled at the end of November 2008 from the outside sub-sectors of the tree in Citrusdal

Creasing severity vs. mineral nutrients in the albedo in the sunny part of fruit			
		r	*P*-value
Creasing severity (0-2)	N	0.7428	0.0907
Creasing severity	P	0.7000	0.1215
Creasing severity	K	0.2278	0.6642
Creasing severity	Ca	-0.3784	0.4594
Creasing severity	Mg	-0.3069	0.5542
Creasing severity	Na	0.2101	0.6895
Creasing severity	Mn	-0.1348	0.7990
Creasing severity	Fe	-0.1547	0.7697
Creasing severity	Cu	0.6325	0.1778
Creasing severity	Zn	0.1464	0.7820
Creasing severity	B	-0.0555	0.9169
Creasing severity vs. mineral nutrients in the albedo in the shady part of fruit			
Creasing severity (0-2)	N	0.4629	0.3564
Creasing severity	P	0.0488	0.9268
Creasing severity	K	0.3704	0.4698
Creasing severity	Ca	-0.1752	0.7398
Creasing severity	Mg	-0.0320	0.9520
Creasing severity	Na	0.3422	0.5067
Creasing severity	Mn	-0.0607	0.9091
Creasing severity	Fe	-0.3920	0.4421
Creasing severity	Cu	0.9109	0.0115
Creasing severity	Zn	-0.0350	0.9476
Creasing severity	B	-0.3926	0.4413

Table 15. Relationship between creasing severity at harvest and macronutrient or micronutrient concentrations in the albedo tissue of 'Washington' navel fruit in the outside (sunny) and inside (shady) part of fruit sampled in mid December 2008 from the outside sub-sectors of the tree in Citrusdal.

Creasing severity vs. mineral nutrients in the albedo in the sunny part of fruit			
		r	*P*-value
Creasing severity (0-2)	N	0.3347	0.5167
Creasing severity	P	0.1581	0.7648
Creasing severity	K	0.8812	0.0203
Creasing severity	Ca	-0.1394	0.7988
Creasing severity	Mg	-0.3780	0.4601
Creasing severity	Na	-0.4940	0.3193
Creasing severity	Mn	-0.9342	0.0064
Creasing severity	Fe	-0.2068	0.6942
Creasing severity	Cu	0.4000	0.4320
Creasing severity	Zn	-0.7683	0.0743
Creasing severity	B	-0.3492	0.4975
Creasing severity vs. mineral nutrients in the albedo in the shady part of fruit			
Creasing severity (0-2)	N	-0.1152	0.8280
Creasing severity	P	0.6276	0.1822
Creasing severity	K	-0.3367	0.5141
Creasing severity	Ca	-0.1710	0.7461
Creasing severity	Mg	-0.7188	0.1075
Creasing severity	Na	-0.5485	0.2598
Creasing severity	Mn	-0.6662	0.1486
Creasing severity	Fe	0.4720	0.3446
Creasing severity	Cu	0.2818	0.5885
Creasing severity	Zn	-0.6312	0.1789
Creasing severity	B	0.1949	0.7113

Table 16. Relationship between creasing severity at harvest and macronutrient or micronutrient concentrations in the albedo tissue of 'Washington' navel fruit in the outside (sunny) and inside (shady) part of fruit sampled at the end of January 2009 from the outside sub-sectors of the tree in Citrusdal.

Creasing severity vs. mineral nutrients in the albedo in the sunny part of fruit			
		r	*P*-value
Creasing severity (0-2)	N	-0.0193	0.9710
Creasing severity	P	-0.8000	0.0560
Creasing severity	K	-0.0501	0.9249
Creasing severity	Ca	0.2046	0.6974
Creasing severity	Mg	0.1240	0.8149
Creasing severity	Na	-0.5394	0.2694
Creasing severity	Mn	-0.2794	0.5918
Creasing severity	Fe	-0.3621	0.4806
Creasing severity	Cu	-0.4339	0.3900
Creasing severity	Zn	-0.4152	0.4130
Creasing severity	B	0.4000	0.4320
Creasing severity vs. mineral nutrients in the albedo in the shady part of fruit			
Creasing severity (0-2)	N	0.5078	0.3037
Creasing severity	P	-0.4140	0.4144
Creasing severity	K	-0.0649	0.9028
Creasing severity	Ca	0.2887	0.5790
Creasing severity	Mg	-0.2044	0.6976
Creasing severity	Na	-0.0800	0.8803
Creasing severity	Mn	0.3131	0.5456
Creasing severity	Fe	0.1851	0.7255
Creasing severity	Cu	0.2246	0.6688
Creasing severity	Zn	-0.4831	0.3317
Creasing severity	B	-0.3624	0.4801

Table 17. Relationship between creasing severity at harvest and macronutrient or micronutrient concentrations in the albedo tissue of 'Washington' navel fruit in the outside (sunny) and inside (shady) part of fruit sampled at the end of February 2009 from the outside sub-sectors of the tree in Citrusdal.

Creasing severity vs. mineral nutrients in the albedo in the sunny part of fruit			
		r	*P*-value
Creasing severity (0-2)	N	0.0933	0.8605
Creasing severity	P	0.1581	0.7648
Creasing severity	K	0.2084	0.6919
Creasing severity	Ca	-0.3664	0.4750
Creasing severity	Mg	-0.7519	0.0847
Creasing severity	Na	-0.3108	0.5489
Creasing severity	Mn	-0.6325	0.1778
Creasing severity	Fe	0.5154	0.2953
Creasing severity	Cu	-	-
Creasing severity	Zn	-0.7593	0.0800
Creasing severity	B	0.4741	0.3422
Creasing severity vs. mineral nutrients in the albedo in the shady part of fruit			
Creasing severity (0-2)	N	0.7890	0.0621
Creasing severity	P	-	-
Creasing severity	K	0.6374	0.1734
Creasing severity	Ca	-0.3912	0.4432
Creasing severity	Mg	-0.6973	0.1236
Creasing severity	Na	-0.2954	0.5697
Creasing severity	Mn	-0.4141	0.4144
Creasing severity	Fe	0.1734	0.7424
Creasing severity	Cu	0.1208	0.8197
Creasing severity	Zn	0.0276	0.9507
Creasing severity	B	0.5175	0.2930

Table 18. Relationship between creasing severity at harvest and macronutrient or micronutrient concentrations in the albedo tissue of 'Washington' navel fruit in the outside (sunny) and inside (shady) part of fruit sampled at the end of March 2009 from the outside sub-sectors of the tree in Citrusdal

Creasing severity vs. mineral nutrients in the albedo in the sunny part of fruit			
		r	*P*-value
Creasing severity (0-2)	N	0.3133	0.8605
Creasing severity	P	0.4000	0.7648
Creasing severity	K	-0.8248	0.6919
Creasing severity	Ca	0.5965	0.4750
Creasing severity	Mg	-0.0555	0.0847
Creasing severity	Na	0.5115	0.5489
Creasing severity	Mn	0.4000	0.1778
Creasing severity	Fe	0.8505	0.2953
Creasing severity	Cu	-0.4000	-
Creasing severity	Zn	0.7856	0.0800
Creasing severity	B	0.6102	0.3422
Creasing severity vs. mineral nutrients in the albedo in the shady part of fruit			
Creasing severity (0-2)	N	-0.4247	0.4013
Creasing severity	P	-	-
Creasing severity	K	-0.7929	0.0599
Creasing severity	Ca	0.5093	0.3021
Creasing severity	Mg	0.1051	0.8429
Creasing severity	Na	0.6476	0.1644
Creasing severity	Mn	0.6410	0.1702
Creasing severity	Fe	0.7231	0.1044
Creasing severity	Cu	0.0569	0.9147
Creasing severity	Zn	0.4365	0.3861
Creasing severity	B	0.5256	0.2841

Table 19. Relationship between creasing severity at harvest and macronutrient or micronutrient concentrations in the albedo tissue of 'Washington' navel fruit in the outside (sunny) and inside (shady) part of fruit sampled at the end of April 2009 from the outside sub-sectors of the tree in Citrusdal.

Creasing severity vs. mineral nutrients in the albedo in the sunny part of fruit			
		r	*P*-value
Creasing severity (0-2)	N	-0.1952	0.7109
Creasing severity	P	0.3162	0.5415
Creasing severity	K	0.7000	0.1215
Creasing severity	Ca	-0.7672	0.0750
Creasing severity	Mg	-0.7683	0.0743
Creasing severity	Na	-0.2763	0.5961
Creasing severity	Mn	-0.3162	0.5415
Creasing severity	Fe	0.1397	0.7918
Creasing severity	Cu	-0.4000	0.4320
Creasing severity	Zn	-0.2774	0.5946
Creasing severity	B	-0.7766	0.0693
Creasing severity vs. mineral nutrients in the albedo in the shady part of fruit			
Creasing severity (0-2)	N	0.0426	0.9361
Creasing severity	P	0.4428	0.3792
Creasing severity	K	0.4055	0.4251
Creasing severity	Ca	-0.4811	0.3340
Creasing severity	Mg	-0.1074	0.8395
Creasing severity	Na	0.0078	0.9882
Creasing severity	Mn	-0.1260	0.8120
Creasing severity	Fe	-0.5360	0.2730
Creasing severity	Cu	-	-
Creasing severity	Zn	-0.4839	0.3308
Creasing severity	B	0.1271	0.8104

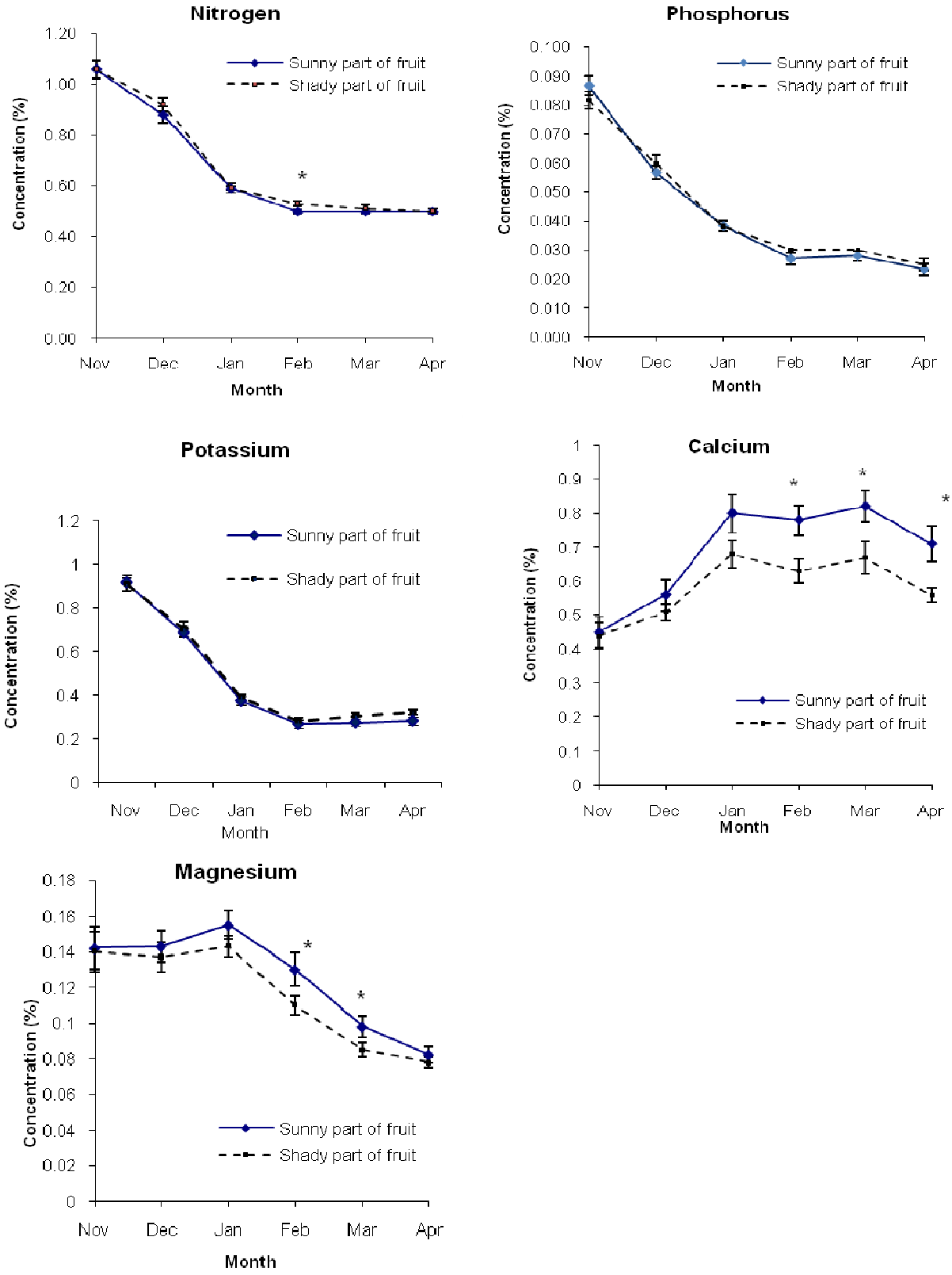

Fig. 1. Albedo macronutrient concentrations of the sunny part and the shady part of 'Washington' navel orange fruit sampled from November 2008 to April 2009 in Citrusdal.

*Significant at $P \leq 0.05$ (Duncan), error bars denote SE (n=6)

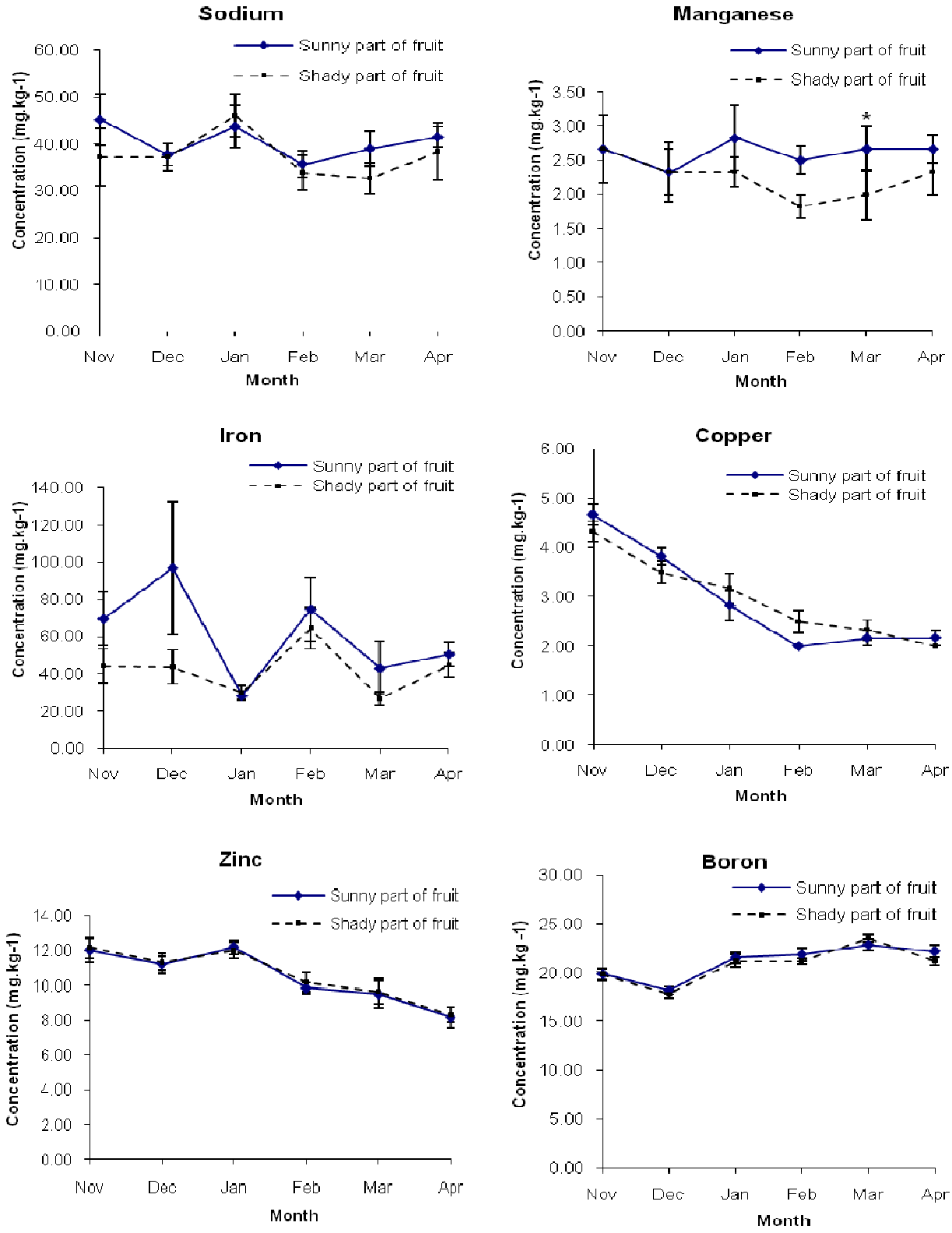

Fig. 2. Albedo micronutrient concentrations of the sunny part and the shady part of 'Washington' navel orange fruit sampled from November 2008 to April 2009 in Citrusdal.

*Significant at $P \leq 0.05$ (Duncan), error bars denote SE (n=6)

4. DETERMINATION OF THE MOST EFFECTIVE TIMING OF GIBBERELLIC ACID (GA_3) APPLICATION TO REDUCE THE INCIDENCE OF CREASING ON 'PALMER' AND 'WASHINGTON' NAVEL ORANGES

ABSTRACT. Creasing is a major problem in the Eastern and Western Cape of South Africa, especially on Navels and Valencia's. The application of gibberellic acid (GA_3) reduces the incidence and severity of creasing and is used commercially to control creasing in most citrus producing countries. However, the use of GA_3 has a negative effect on the fruit rind colour development. In order to determine the most effective application timing of GA_3 with the least negative effect on fruit rind colour development, two trials were conducted in Citrusdal over a period of two years. Messenger® was also investigated to evaluate its effect on the incidence and severity of creasing. 'Palmer' navel orange trees were used in trial one (2008) and 'Washington' navel orange trees were used in trial two (2009). GA_3 (10 $mg \cdot L^{-1}$) was applied during specific months of stage II of citrus fruit development in late November, December and mid January in 2008 and in mid December, January and early February in 2009. Messenger® (0.03 $mg \cdot L^{-1}$), was applied multiple times at all the GA_3 application timings mentioned above only in 2008. In both years, creasing incidence and creasing severity was significantly reduced by the application of GA_3, irrespective of the timing of application. The mid December applications (20 Dec. 2007 and 18 Dec. 2008) were most effective in reducing creasing incidence, although they were not significantly different from the other GA_3 application timings. A significant delay in fruit rind colour development at harvest was observed. In 2008, fruit treated on 14 January had the weakest fruit rind colour development at harvest compared to the earlier applications in December and mid November while in 2009, a similar delay in fruit rind colour development was observed for all the GA_3 application timings, except for fruit treated on 23 January. Messenger® did not reduce creasing incidence and creasing severity. Therefore, to significantly reduce the incidence and severity of creasing GA_3 at 10 $mg \cdot L^{-1}$ can be applied from mid November until mid January.

Introduction

Creasing is a pre-harvest physiological disorder usually observed at post-colour break (Bar-Akiva, 1975). Early onset of senescence (Monselise et al., 1976) or expression of rapidly progressing senescence (Monselise, 1973) is associated with the development of creasing. Hence, creasing is characterized as one of the rind disorders associated with aging (Coggins, 1973). Such disorders are reduced by preharvest application of gibberelic acid (GA_3) (Coggins, 1973; Monselise, 1973, 1979). GA_3 appears to impede the initial development of creasing through its action of decreasing the pectin methyl esterase activity (Jona et al., 1989), which is unusually high in affected fruit (Jones et al., 1967), and increases the firmness and strengthens the rind of the fruit (Coggins, 1969; Gambetta et al., 2000).

The most effective GA_3 concentration is 10 $mg \cdot L^{-1}$ and 20 $mg \cdot L^{-1}$ depending on the location, time of application and severity of creasing. Tugwell et al. (1996) observed that a high volume application of 20 $mg \cdot L^{-1}$ GA_3 concentration was effective in controlling creasing under South Australian conditions. Gilfillan et al. (1980, 1981) recommended a concentration of 10 $mg \cdot L^{-1}$ GA_3 concentration on Navels under South African conditions while Monselise et al. (1976) recommended 20 $mg \cdot L^{-1}$ GA_3 concentration on Valencia's under Israeli conditions. In addition, the effectiveness of GA_3 can be increased by acidification of the spray mixture to pH 4.0 (Gambetta et al., 2000; Gilfillan and Cutting, 1992; Greenberg and Goldschmidt, 1988; Greenberg et al., 1992; Tugwell et al., 1996).

During active rind growth of stage II and stage III of fruit growth, GA_3 application effectively reduces creasing (Monselise, 1973). Hence, earlier applications when fruitlets are 30-55 mm in diameter were observed to effectively reduce creasing incidence (Bevington, 1973; Gambetta et al., 2000; Gilfillan et al., 1980, Gilfillan et al., 1981; Monselise, 1973; Monselise et al., 1976; Tugwell et al., 1996). Later applications when fruitlets were larger than 65 mm were also effective but it resulted in a strong negative effect on fruit rind colour development (Gambetta et al., 2000; Gilfillan et al., 1974; Gilfillan et al., 1980; Gilfillan et al., 1981; Monselise et al., 1976; Monselise, 1979). Therefore, the objective of

this study was to determine the most effective application timing of GA_3 with the least negative effect on fruit rind colour development and Messenger® was also evaluated for its effect on creasing incidence and creasing severity. Messenger is a harpin is a protein produced in nature by certain bacterial plant pathogens and when harpin is applied to plants it activates a plant's natural growth and defence mechanisms (Jones, 2001)

Materials and methods

Plant material. The experiments were conducted on navel orange trees over a period of two years at two different sites with a history of severe creasing incidence. 'Palmer' navel orange trees were used in the first year (2008) in a commercial orchard located in Citrusdal (32°36'S, 19°1'E) in the Western Cape, South Africa. Tree spacing was 6m between rows and 3m in rows and the row direction of the orchard was north to south. The field trial consisted of a randomized complete block design with six treatments and eight single tree replicates per treatment.

In the second year (2009), 'Washington' navel orange trees planted at a spacing of 6m between rows and 6m in rows in a north to south row direction were used. This orchard also has a history of severe creasing incidence and is situated in Citrusdal in the Western Cape, South Africa. The experiment was laid out as a randomized complete block design with five treatments and six single tree replicates per treatment.

Treatments. Gibberellic acid (GA_3) at 10 $mg \cdot L^{-1}$ was applied at four different application timings. Breakthru was added as a wetter at 0.05 $mL \cdot L^{-1}$. In the first year of the study, treatments were applied at the end of November (26 Nov. 2007), at the beginning of December (11 Dec. 2007), mid December (20 Dec. 2007), and in mid January (14 Jan. 2008). Messenger® (0.03 $mg \cdot L^{-1}$) was applied multiple times at all four dates mentioned above. In the second year treatments consisted of only applications of GA_3 in mid December (18 Dec. 2008), at the beginning of January (12 Jan. 2009), at the end of January (23 Jan. 2009) and at the beginning of February (2 Feb. 2009).

Measurements and data collection. At commercial harvest (14 May 2008 and 5 May 2009), an on-tree evaluation of creasing severity and fruit rind colour was carried out. Creasing severity was evaluated on a score of 0 to 4; the orange was divided into four equivalent imaginary spheres. If no sphere was creased it was designated a zero, for a sphere that showed creasing the score was noted from 1 (one sphere) to 4 (all spheres of the fruit). Fruit rind colour was determined based on the CRI fruit rind colour chart (Set No: 34) for oranges (Appendix 1), with a range between one and eight, one being completely orange and eight being green. Creasing incidence as a percentage of fruit evaluated was also calculated for the on-tree evaluation.

In 2008, fourty fruit per single tree were evaluated while twenty fruit per single tree were evaluated in 2009. Ten fruit (2008) or five fruit (2009) per single tree replicate were evaluated at random from the outside of the tree at eye-sight level from each of the four sectors of the tree; north, south, east and west.

Statistical analysis. Analysis of variance was performed using the computer program SAS (Statistical Analysis System) Enterprise Guide 3. Duncan's multiple range test at P= 0.05 was used to test the treatment effects. All percentages data were arcsine transformed to degrees asin√ before the statistical calculations were made.

Results

In the first year (2008) creasing incidence and creasing severity were <25% and < 0.50 respectively (Table 1). Creasing incidence and creasing severity was significantly reduced by the application of gibberellic acid (GA_3), irrespective of the timing of application (Table 1). Therefore, in this study, the earlier mid November application (26 Nov. 2007) was as effective as the December applications (11 Dec. 2007 and 20 Dec. 2007) and the later mid January application (14 Jan. 2008) in reducing creasing incidence and creasing severity. The mid December application (20 Dec. 2007) was the most

effective in reducing creasing incidence,compared to the other treatments although it was not significantly different from the other GA_3 application timings (Table 1). Messenger® did not reduce creasing incidence or creasing severity compared to the control (Table 1). Fruit rind colour was significantly delayed by all the GA_3 treatments as well as by Messenger® (Table 1). Fruit treated on 14 Jan. 2008 had the poorest fruit rind colour development at harvest compared to the earlier applications in December and mid November.

[Table 1]

Creasing incidence and creasing severity was ranked from the highest to the lowest as follows: north > south > east = west. However, the incidence of creasing was not significantly different between the southern, western and eastern sectors while creasing severity was greater in the northern sector (0.62) and southern sector (0.38) of the trees compared to the other sectors (Table 2). In addition, fruit rind colour differed significantly amongst the sectors and was ranked from the best to worse as follows: south> east> north> west (Table 2). However, during 2008, a weak negative correlation (P = 0.0001) between fruit rind colour and creasing incidence (r= -0.30) was observed (Table 3).

[Table 2 and 3]

In the second year (2009), creasing incidence and creasing severity on 'Washington' navel oranges were 10% and 0.16 respectively (Table 4). Fruit were however, harvested a week earlier than the normal commercial picking time due to the tendency of creasing incidence to be more pronounced as the season progresses. Even though, very little creasing occurred, the application of GA_3 significantly reduced creasing incidence and creasing severity, irrespective of the timing of application (Table 4). The mid December application (18 Dec. 2008) was the most effective in reducing creasing incidence, although it was not significantly different from other GA_3 application timings. Fruit rind colour development was delayed in all the GA_3 application timings, except for fruit treated on 23 Jan. 2009 (Table 4). In 2009 a very weak non significant negative correlation between fruit rind colour and creasing incidence (r = -0.11) was observed (Table 5).

[Table 4 and 5]

Discussion

GA_3 is used commercially to significantly reduce the incidence of creasing in most citrus producing countries (Bevington, 1973; Coggins, 1969; Embleton et al., 1973; Gilfillan et al., 1980, 1981; Jones et al., 1967; Monselise et al., 1976; Tugwell et al., 1996). As expected, in our studies the use of GA_3 significantly reduced creasing incidence and creasing severity. The earlier GA_3 application in mid November was as effective as the later GA_3 applications in December, January and early February, which concur with Gilfillan et al. (1980). On the contrary, in another study Gilfillan et al. (1981) observed that earlier GA_3 applications in November were less effective than later applications in December, January and February in reducing creasing incidence in South Africa. Contrary to this, Gambetta et al. (2000) observed that later GA_3 applications in April were less effective than the early GA_3 applications in January or February GA_3 applications in Uruguay. Similarly, Bevington (1973) in Australia (New South Wales) observed that later GA_3 applications in June were less effective than the early GA_3 applications in May in reducing creasing incidence.

However, in this study the mid December GA_3 applications were the most effective in reducing creasing incidence, although they did not significantly differ from the other treatments. Similarly, Gilfillan et al (1981) also noted that mid December to mid January (70-100 days after anthesis) applications give the best results under South African conditions. This is usually the stage when the fruit diameter is 30 to 55 mm and previous researchers also reported the effectiveness of GA_3 sprays at this stage of fruit growth (Bevington, 1973; Gambetta et al., 2000; Gilfillan et al., 1974; Gilfillan et al., 1980; Monselise et al., 1976; Monselise, 1979; Tugwell et al., 1996).

A significant delay in fruit rind colour development at harvest was observed in both years. In the first year, the later GA_3 application in mid January had the weakest fruit rind colour development at harvest, compared to the earlier applications in mid November and December while in the second

year, a similar delay in fruit rind colour development was observed for all GA_3 application timings except for fruit treated at the end of January. Generally, in citrus the application of GA_3 results in a delay in fruit rind colour development (Coggins and Jones 1977; Coggins, 1969, 1981; Goldschmidt and Eilati, 1970; Monselise et al., 1976). This effect is more pronounced when fruitlets are larger than 65 mm compared to when fruitlets are 30 to 55 mm in diameter (Gambetta et al., 2000; Gilfillan et al., 1974; Gilfillan et al., 1980; Gilfillan et al., 1981; Monselise et al., 1976; Monselise, 1979).

In this study the relationship between creasing incidence and fruit rind colour development could not be established as shown by the very weak correlations between the fruit rind colour and creasing incidence. However, Embleton et al. (1973) reported that creasing incidence is inversely related to the fruit rind colour at harvest. Creasing severity was greater in fruit in the north side of the GA_3 treated trees, with the least creasing in the south, west and eastern side of the GA_3 treated tree. Contrary to this, Gilfillan et al (1981) observed more creasing in the south side than in the north side of the GA_3 sprayed trees.

In conclusion, creasing incidence and creasing severity was reduced by the application of GA_3, irrespective of the timing of application. However, the mid December GA_3 applications gave the best result. Therefore, it is possible that mid December is the best time to apply GA_3 in order to reduce creasing incidence in South Africa. A delay in fruit rind colour development at harvest was observed with the poorest fruit rind colour development in the later mid January GA_3 application. Therefore, in order to achieve significant reductions in creasing incidence, GA_3 can be applied from mid November until mid January and GA_3 spray applications later than mid January should be avoided, due to a stronger inhibition of the fruit rind colour development.

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Table 1. On-tree evaluation of GA_3 application timings on the creasing incidence, creasing severity and fruit rind colour of 'Palmer' navel oranges in Citrusdal in 2008.

Treatment	**Creasing incidence**	**Creasing severity**	**Fruit rind colour**
	---%---	*--0-4[x]--*	*--1-8[y]--*
1. Control	24.37 a[z]	0.52 a	2.53 d
2. GA_3 (26 Nov. 2007)	15.48 bc	0.32 b	2.89 c
3. GA_3 (11 Dec. 2007)	17.18 bc	0.30 b	3.07 ab
4. GA_3 (20 Dec. 2007)	12.81 c	0.28 b	3.08 ab
5. GA_3 (14 Jan. 2008)	15.31 bc	0.36 b	3.12 a
6. Messenger® (26 Nov. 2007, 11 Dec. 2007, 20 Dec. 2007 and 14 Jan.2008)	20.63 ab	0.42 ab	2.91 bc
P-value	0.0209	0.0053	0.0001

[z]Means with the same letter are not significantly different at the 5% level (Duncan)
[y] 1-8 (1= orange, 8= green) CRI fruit rind colour chart (Set No: 34) for oranges
[x] 0-4 (0=0%, 1= 25%, 2=50%, 3=75%, 4=100%)

Table 2. The relationship of the bearing position of fruit on a tree and creasing incidence, creasing severity and fruit rind colour of 'Palmer' navel oranges in Citrusdal in 2008.

Treatment	**Creasing incidence**	**Creasing severity**	**Fruit rind colour**
	---%---	*--0-4[x]--*	*--1-8[y]--*
1. North	25.42 a[z]	0.62 a	3.17 b
2. South	18.33 ab	0.38 b	2.29 d
3. East	13.62 b	0.24 c	2.90 c
4. West	13.12 b	0.22 c	3.38 a
P-value	0.0062	0.0043	0.0001

[z]Means with the same letter are not significantly different at the 5% level (Duncan)
[y] 1-8 (1= orange, 8= green) CRI fruit rind colour chart (Set No: 34) for oranges
[x] 0-4 (0=0%, 1= 25%, 2=50%, 3=75%, 4=100%)

Table 3. Relationship between fruit rind colour and creasing incidence (%) of 'Palmer' navel oranges sampled in Citrusdal in 2008

Fruit rind colour vs. creasing %	**2008**	
	r	*P*-value
Fruit rind colour	-0.30	0.0001

Table 4. On-tree evaluation of GA_3 application timings on creasing incidence, creasing severity and fruit rind colour of 'Washington' navel oranges in Citrusdal in 2009.

Treatment	**Creasing incidence**	**Creasing severity**	**Fruit rind colour**
	---%---	*--0-4[x]--*	*--1-8[y]--*
1. Control	10.00 a[z]	0.16 a	4.65 b
2. GA_3 (18 Dec. 2008)	0.00 b	0.00 b	5.08 a
3. GA_3 (12 Jan. 2009)	0.83 b	0.01 b	4.96 a
4. GA_3 (23 Jan. 2009)	2.50 b	0.04 b	4.63 b
5. GA_3 (2 Feb. 2009)	0.83 b	0.01 b	4.98 a
P-value	0.0346	0.0001	0.0001

[z]Means with the same letter are not significantly different at the 5% level (Duncan)
[y] 1-8 (1= orange, 8= green) CRI fruit rind colour chart (Set No: 34) for oranges
[x] 0-4 (0=0%, 1= 25%, 2=50%, 3=75%, 4=100%)

Table 5. Relationship between fruit rind colour and creasing incidence (%) of 'Washington' navel oranges sampled in Citrusdal in 2009.

Fruit rind colour vs. creasing %	**2009**	
	r	*P*-value
Fruit rind colour	-0.11	0.5522

5. THE EFFECTS OF DIFFERENT PLANT GROWTH REGULATORS AND OTHER PRODUCTS ON THE INCIDENCE AND SEVERITY OF CREASING OF 'BAHIANINHA' AND 'PALMER' NAVEL ORANGES

ABSTRACT. Creasing is a physiological disorder that affects the albedo of citrus fruit causing creases on the surface of the fruit. The physiological basis of creasing development is unknown, but it is suggested that all the factors which limit the enlargement of the outer layers of the fruit while enlargement of the inner layers is favoured will result in creasing development. Early onset of senescence is also associated with creasing development. Hence, extension of the period of cell division or delaying senescence could be a key in the control of creasing incidence. To evaluate the effectiveness of CPPU [N-(2-chloro-4-pyridyl)-N.-phenylurea], MaxCel® (6-Benzyladenine), AVG (aminoethoxyvinylglycine) and CPPU in combination with calcium in relation to the incidence and severity of creasing, trials were conducted on 'Bahianinha' (2008) and 'Palmer' (2009) navel orange trees. During 2008, individual fruit were sprayed after the physiological fruit drop. In 2009 treatments were applied as a stem application and to whole shoots at petal drop. In 2008, creasing severity was significantly reduced by the application of CPPU, MaxCel® and CPPU in combination with calcium. Creasing incidence was also reduced by the treatments, although it did not significantly differ from the control. The applications of MaxCel® and the mixture of CPPU and calcium resulted in a significant delay in fruit rind colour development as well as significantly reduced fruit growth and fruit diameter. AVG did not significantly reduce the incidence and severity of creasing. In 2009, low creasing incidence occurred, hence treatment effects on the incidence and severity of creasing could not be established, but stem applications of MaxCel®, CPPU and the mixture of CPPU and calcium resulted in a significantly better fruit rind colour development at harvest. Spraying MaxCel® on the shoots and the fruit dip treatment of AVG significantly increased the number of fruit at harvest compared to the control. The results showed that cytokinins have the potential to reduce creasing incidence and further studies on application and uptake efficiency of cytokinis are recommended.

Introduction

Creasing, a physiological disorder that affects the albedo of citrus fruit, is normally detectable at maturity (Gambetta et al., 2000; Jona et al., 1989) and tends to increase as fruit matures (du Plessis and Maritz, 2004; Nagy et al., 1982). It could be surmised that the potential of the albedo cells to expand and accommodate cell enlargement, after cell division, has ceased and weaknesses of the cell wall connections are critical for creasing development (Holtzhausen, 1981; Storey and Treeby, 1994). Therefore, weaknesses in the middle lamella which connect cells are thought to be associated with the early onset of senescence (Monselise, 1973; Monselise et al., 1976).

Extension of the period of cell division or strengthening of the cohesion at the middle lamella of adjacent albedo cells or delaying the early onset of senescence could be a key in the control of creasing. For instance, all the naturally occurring cytokinins have the ability to stimulate cell division in plants (Salisbury, and Ross, 1992; Van Staden and Cook, 1986). In addition adequate calcium concentrations are needed in plant cells to form calcium pectates in the middle lamella of the cell plate of daughter cells (Hopkins and Huner, 2004; Taiz and Zeiger, 2002) and thus the ability to strengthen the middle lamella and reduce the development of creasing (Storey and Treeby, 2002; Storey et al., 2002). Plant hormones play a vital role in the rate of cellular senescence and ethylene-inhibiting products could have a potential to reduce creasing through their ability to delay senescence.

Aminoethoxyvinylglycine (AVG) is a known inhibitor of ethylene (Vizzotto et al., 2002), has been used to retard preharvest drop in apples (Bramlage et al., 1980; Byers, 1997; Brackmann and Waclawovsky, 2001; Williams, 1980). Preliminary studies showed that preharvest application of AVG on citrus reduces the early onset of senescence and also the incidence of creasing (Gonzalez and Lovatt, 2004). CPPU (N-(2-chloro-4pyridyl)-N-phenyl urea) and MaxCel® (6-benzyladenine) exhibit cytokinin-like properties when applied to plants. In citrus, the primary physiological effect of CPPU is an increase in the fruit size (Erner et al., 2004).

Thus, the aim of the study was to evaluate the effectiveness of CPPU and MaxCel® alone and CPPU in combination with calcium to reduce incidence and severity of creasing. The effect of AVG on creasing incidence was also investigated. Cytokinins (CPPU and MaxCel®) were applied to stimulate cell division in fruitlets while calcium was also added to strengthen cell wall connections in the albedo tissue. AVG was applied to inhibit ethylene synthesis and thereby, delaying the normal aging of the rind.

Materials and methods

Plant material and layout. Navel orange trees were used for this study in 2008 and 2009. The trials were conducted in commercial orchards in Citrusdal (32°36'S, 19°1'E) in the Western Cape, South Africa. In the first year (2008) one trial was carried out on 'Bahianinha' navel orange trees planted at a spacing of 5m between rows and 2.5m in rows in an east to west row orientation. The experiment was laid out as a randomized complete block design with eight single tree replicates per treatment. Ten fruit per tree were tagged randomly around the tree for treatments.

In the second year (2009) two trials were conducted on 'Palmer' navel orange trees. Tree spacing was 6m between rows and 3m in rows and the row direction of the orchard was north to south. The first trial was laid out as a randomized block design with four treatments and eight single tree replicates per treatment. In the second trial ten bearing shoots per tree were tagged and treated. All the treatments were replicated on twelve single tree replicates.

Treatments

In the first year (2008) the treatment solutions consisted of CPPU (10 $mg \cdot L^{-1}$), MaxCel® (190 $mg \cdot L^{-1}$), AVG (38 $mg \cdot L^{-1}$) and a mixture of CPPU (10 $mg \cdot L^{-1}$) and chelated calcium (84 $g \cdot L^{-1}$). Tagged fruit were individually sprayed using a handheld spray bottle. All treatments were applied after the physiological fruit drop on 30 Nov. 2007. In 2009, treatment solutions also consisted of CPPU (10 $mg \cdot L^{-1}$), MaxCel® (190 $mg \cdot L^{-1}$) and a mixture of CPPU (10 $mg \cdot L^{-1}$) and chelated calcium (84 $g \cdot L^{-1}$) for

the first trial. These treatments were applied as a stem application using a brush at petal drop on 29 Oct. 2008. In a second trial in 2009 treatments consisted of GA_3 (10 mg·L^{-1}), CPPU (10 mg·L^{-1}), AVG (38 mg·L^{-1}) and a mixture of CPPU (10 mg·L^{-1}) and chelated calcium (84 g·L^{-1}). Five out of the ten tagged shoots were sprayed with a treatment solution using a spray bottle, four shoots were dipped in the treatment solution and one shoot was left as the untreated control. These treatment solutions were applied at petal drop on 29 Oct. 2008.

Fruit growth. In 2008 the fruit diameter was measured throughout the season until harvesting on the following dates, 30 Nov. 2007, 17 Jan. 2008, 23 Feb. 2008, 28 Mar. 2008 and 10 May 2008 using an electronic calliper (CD-6″ C, Mitutoyo Corp, Toyko, Japan). The rate of fruit growth was calculated by subtracting the initial fruit diameter from the final fruit diameter divided by the number of days in between.

Measurements and data collection. In the first year, (2008) the fruit were harvested on 10 May 2008. Creasing severity was evaluated on a score of 0 to 4; the orange was divided into four equivalent imaginary spheres. If no sphere was creased it was designated a zero, for a sphere that showed creasing the score was noted from 1 (one sphere) to 4 (all spheres of the fruit). Fruit rind colour was determined based on the CRI fruit rind colour chart (Set No: 34) for oranges (Appendix 1), with a range between one and eight, one being completely orange and eight being wholly green. The fruit diameter and rind thickness on the outside (sunny) part of each fruit was measured using an electronic calliper (CD-6″ C, Mitutoyo Corp, Toyko, Japan).

In the second year (2009) an on-tree evaluation of creasing severity and fruit rind colour was carried out at commercial harvest on 18 May 2009. Five fruit (2009) per single tree replicate were evaluated at random from the outside of the tree at eye-sight level on each of the four sectors of the tree; north, south, east and west. Creasing severity and fruit rind colour were evaluated similar to the first year of the study (2008). Creasing incidence as a percentage of the fruit evaluated was also calculated.

Statistical analysis. Analysis of variance was performed using the computer program SAS (Statistical Analysis System) Enterprise Guide 3. Duncan's multiple range test at P= 0.05 was used to test the treatment effect. All percentages were arcsine transformed to degrees asin√ before the statistical calculations were made.

Results

Creasing incidence (50.38 %) and creasing severity (1.18) on 'Bahianinha' navel oranges was high in 2008 (Table 1). The application of MaxCel® and CPPU and CPPU in combination with calcium reduced creasing incidence, although not significantly (Table 1). Creasing severity was significantly reduced by all the treatments, except for AVG with MaxCel® and CPPU giving the best results (Table1). Therefore, in this study MaxCel® and CPPU applied alone was the most effective in reducing creasing severity. MaxCel® and CPPU in combination with calcium resulted in a significant delay in fruit rind colour development with the poorest fruit rind colour development on fruit treated with the mixture of CPPU and calcium (Table 1). Trees were water stressed at one occasion during the course of the experiment. Rind thickness was not significantly affected by any of the treatments (Table 1). Fruit treated with the mixture of CPPU and calcium resulted in significantly smaller fruit at harvest. Fruit growth rate and final fruit size was significantly reduced on fruit treated with CPPU alone and with CPPU in conjunction with calcium (Table 2).

In 2009 in the first trial, very little creasing occurred at harvest on 'Palmer' navel oranges in Citrusdal. Therefore, the treatment effects of stem application of MaxCel®, CPPU and a mixture of CPPU and chelated calcium on creasing incidence and creasing severity could not be established. MaxCel®, CPPU, and CPPU in combination with calcium applied as stem applications resulted in a significantly better fruit rind colour development at harvest (Table 3).

[Table 3]

In the second trial, no creasing developed at harvest on 'Palmer' navel fruit in Citrusdal, hence the effect of spraying shoots and dipping shoots with GA_3, MaxCel®, CPPU, AVG and a mixture of

CPPU and chelated calcium on creasing incidence and creasing severity could not be established. Spraying MaxCel® on the shoots and the fruit dip treatment of AVG significantly increased the number of fruit at harvest when compared to the control. Fruit rind colour was not affected by any of the spray applications or fruit dip applications (Table 4).

[Table 4]

Discussion

In the first year of the study, CPPU and MaxCel® applications were the most effective in reducing creasing severity. However, the effect of these treatments could not be established in the second year, because very little or no creasing developed. Preharvest application of AVG, an ethylene inhibitor, did not reduce creasing incidence, which was contrary to what was observed in preliminary trials in California (Gonzalez and Lovatt, 2004). However, it could be possible that the extension of cell division or delaying senescence, could be a key in the control of creasing, as it is known that cytokinin-like products act by stimulating additional cell division (Salisbury, and Ross, 1992; Van Staden and Cook, 1986) and ethylene inhibitors delay senescence in plants (Bregoli et al., 2002).

In the first year, fruit sprayed with MaxCel®, and CPPU in combination with calcium resulted in a significant delay in fruit rind colour. However, in the second year stem application of MaxCel®, CPPU and CPPU in combination with calcium resulted in a significantly better fruit rind colour development at harvest, while fruit rind colour development of fruit on trees where whole shoots were sprayed or dipped in the treatment solution was not affected. Monselise (1979) suggested that exogenous cytokinins in citrus may prevent degreening of fruit, as in many instances cytokinins could act similarly to gibberellins in this regard. In citrus, the primary physiological effect of CPPU is an increase in the fruit size (Erner et al., 2004) and an increase in fruit size or rind thickness could be expected. Contrary to this, fruit were small at harvest and the rind thickness was not affected by any of the treatments which could be due to water stress during the course of the experiment.

In conclusion, localised fruit applications of MaxCel® and CPPU alone or CPPU in combination with calcium after physiological fruit drop significantly reduced creasing incidence at harvest. The mixture of CPPU and calcium was not as effective as the single application of CPPU. Hence, cytokinins could potentially be used to reduce the incidence and severity of creasing. However, studies on methods of application and uptake efficiency of cytokinins are required. Additionally, it is important to assess the effectiveness of stem application and fruit dip treatments of MaxCel® and CPPU alone or CPPU in combination with calcium, because little or no creasing occurred in the second year of the study and these treatment effects on creasing incidence could not be established.

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Table 1. The effects localised fruit applications of CPPU, MaxCel®, aminoethovinylyglycine (AVG) and calcium on creasing incidence, creasing severity, fruit rind colour, fruit diameter and rind thickness of 'Bahianinha' navels in Citrusdal in 2008.

Treatment	**Creasing incidence**	**Creasing severity**	**Fruit rind colour**	**Fruit diameter**	**Rind thickness**
	---%---	*--0-4[x]--*	*--1-8[y]--*	*--mm--*	*--mm--*
1. Control	50.38	1.18 a[z]	4.59 cd	55.18 a	4.98
2. MaxCel®	31.25	0.55 c	4.88 ab	55.35 a	4.93
3. CPPU	30.00	0.45 c	4.86 bc	53.15 ab	5.10
4. AVG	54.50	1.01 ab	4.56 d	55.06 a	5.04
5. CPPU + Chelated calcium	42.25	0.77 bc	5.14 a	52.57 b	4.95
P-value	0.1467	0.0001	0.0001	0.0025	0.8411

[z]Means with the same letter are not significantly different at the 5% level (Duncan)
[y] 1-8 (1= orange, 8= green) CRI fruit rind colour chart (set No: 34) for oranges
[x] 0-4 (0= 0%, 1=25%, 2=50%, 3= 75%, 4= 100%)

Table 2. Fruit growth and final fruit size of 'Bahianinha' navel fruit treated with CPPU, MaxCel®, aminoethovinylyglycine (AVG) and calcium in Citrusdal in 2008.

Treatment	**Rate of fruit growth**	**Final fruit size**
	--mm/day--	*----mm----*
1. Control	0.173 ab	53.30 a
2. MaxCel®	0.174 a	53.08 a
3. CPPU	0.158 cd	51.24 b
4. AVG	0.164 bc	52.94 a
5. CPPU + Chelated calcium	0.153 d	50.65 b
P-value	0.0001	0.0024

[z]Means with the same letter are not significantly different at the 5% level (Duncan)

Table 3. The effects of stem applications of CPPU, MaxCel® and calcium on creasing incidence, creasing severity and fruit rind colour of 'Palmer' navels in Citrusdal in 2009.

Treatment	**Creasing incidence**	**Creasing severity**	**Fruit rind colour**
	---%---	*--0-4[x]--*	*--1-8[y]--*
1. Control	0.00	0.00 b[z]	4.25 a
2. MaxCel®	6.25	0.04 ab	3.87 b
3. CPPU	3.75	0.06 a	3.87 b
4. CPPU + Chelated calcium	1.25	0.01 b	3.94 b
P-value	0.1628	0.0270	0.0008

[z]Means with the same letter are not significantly different at the 5% level (Duncan)
[y] 1-8 (1= orange, 8= green) CRI fruit rind colour chart (set No: 34) for oranges
[x] 0-4 (0= 0%, 1=25%, 2=50%, 3= 75%, 4= 100%)

Table 4. The effects of localised fruit spray applications and fruit drip treatments of GA_3, MaxCel®, CPPU, aminoethovinylglycine (AVG) and calcium on the incidence of creasing, number of fruit and fruit rind colour of 'Palmer' navels in Citrusdal in 2009.

Treatment	**Creasing incidence**	**Number of fruit**	**Fruit rind colour**
	--0-4[x]--	*--no.--*	*--1-8[y]--*
1. Control	0.00	1.17 bc[z]	4.83
2. Spray bottle applications of GA_3	0.00	1.67 abc	4.50
3. Spray bottle applications of MaxCel®	0.00	3.17 a	4.25
4. Spray bottle applications of CPPU	0.00	0.75 c	5.14
5. Spray bottle applications of AVG	0.00	2.58 ab	4.40
6. Spray bottle applications CPPU + Chelated calcium	0.00	0.58 c	5.00
7. Fruit drip treatment of MaxCel®	0.00	2.08 abc	4.56
8. Fruit dip treatment of CPPU	0.00	1.00 bc	3.60
9. Fruit dip treatment of AVG	0.00	2.92 a	4.36
10. Fruit dip treatment of CPPU + Chelated calcium	0.00	1.44 abc	5.14
P-value	-	0.0003	0.1438

[z]Means with the same letter are not significantly different at the 5% level (Duncan)
[y] 1-8 (1= orange, 8= green) CRI fruit rind colour chart (set No: 34) for oranges
[x] 0-4 (0= 0%, 1=25%, 2=50%, 3= 75%, 4= 100%)

6. EFFECT OF THE MANIPULATION OF LIGHT LEVELS, CARBOHYDRATE, AND MINERAL NUTRIENT ALLOCATION IN THE TREE ON CREASING INCIDENCE

ABSTRACT. Creasing is a pre-harvest physiological disorder caused by separation of cells at the middle lamella of albedo tissues. Although the contributing factors for creasing development are known, the physiological basis for its development is still unresolved. To further understand the physiology of creasing development, light manipulation techniques viz. covering leaves behind fruit with a shade cloth, pruning to remove shading branches, covering fruit with brown paper bags and carbohydrate allocation manipulations such as removing leaves from behind fruit, scoring scaffold branches and hand thinning to one fruit per shoot were assessed over a two year period. All treatments were carried out after physiological fruit drop at the end of November in year one and early December in year two. 'Washington' navel orange trees in Citrusdal were used. In both years creasing incidence and creasing severity was not significantly affected by any of the treatments except for the significance of creasing severity, in the first year, by covering fruit with brown paper bags. In the first year, covering leaves behind the fruit with a shade cloth and covering fruit with brown paper bags had a negative effect on fruit rind colour, while in the second year, only hand thinning had a negative effect on fruit rind colour and scoring scaffold branches resulted in a significantly better fruit rind colour at harvest. Negative weak correlations between rind thickness or fruit diameter and creasing incidence or creasing severity were observed. Covering leaves behind fruit with a shade cloth or covering fruit with brown paper bags significantly increased N, P, K and Mn concentrations in the albedo tissue. However, very weak correlations between creasing incidence or creasing severity and mineral nutrients in the albedo tissue were observed at harvest. These results indicate that light levels on the fruit could influence creasing severity and most likely the distribution of mineral nutrients in the albedo tissue.

Introduction

Creasing is a pre-harvest physiological disorder of citrus, of which the physiological basis is unknown. The potential of the albedo cells to expand and accommodate cell enlargement after cell division has ceased (Holtzhausen, 1981; Storey and Treeby, 1994) and the ability of the cell wall material (pectin or plasmallema) to cement adjoining cell walls together is critical in creasing development (Storey and Treeby, 1994).

Carbohydrates are thought to be related to the metabolism and the formation of pectins (Bower, 2004) and hence could play a significant role in creasing development. There are various ways to manipulate carbohydrate allocation in a citrus tree. In general girdling at or after physiological fruit drop during active fruit growth removes competition from the roots (Cohen, 1981; Goren et al., 2003; Li et al., 2003; Mataa et al., 1998) and increases allocation of sugars to the tree canopy (Goren et al., 2003; Iglesias, et al., 2004). Changing the leaf: fruit ratio by fruit thinning has an influence on carbohydrates allocated to a fruit because carbohydrates availability to any particular fruit is dependent upon the carbohydrate sources as well as the number of competitive sinks (Erner et al., 2004). In addition, the partial defoliation of 60 to 75 % of citrus leaves promotes fruitlet abscission because of increased carbohydrate allocation to the fruit (Iglesias et al., 2004).

Light levels within the canopy also plays a significant role in creasing development and it is known that the shady side of the tree has a higher incidence of creasing (Fourie and Joubert, 1957; Le Roux and Crous, 1938). On any given fruit, creasing also develops first on the side towards the trunk (inside) and thus is less severe on the exposed side (outside) of the fruit (Gambetta et al., 2000; Jones et al., 1967). Light has an influence on mineral nutrient allocation in the tree canopy (Cronje, 2009) and a higher concentration immobile nutrients was observed in the rind of outside fruit than in the rind of inside fruit. Mobile nutrients were observed to occur at a higher concentration in rind of inside fruit than outside fruit (Kruger et al., 2005).

The aim of the study was to provide an insight on the role of light levels in the tree canopy and the effect of carbohydrate manipulation techniques in relation to creasing incidence and the albedo mineral concentration. This will help in developing a pruning strategy as a control measure for creasing.

Material and methods

Plant material. The study was conducted over a two-year period in an orchard with a history of severe creasing incidence. 'Washington' navel orange trees were used for the two years, 2008 and 2009. The orchard planted in a north to south/row orientation is located in Citrusdal (32°36'S, 19°1'E) in the Western Cape, South Africa, with 6 m tree spacing between rows and 6m in rows. The experiment was laid out as a randomized complete block design with eight single tree replicates per treatment. Ten fruit bearing shoots per tree were tagged randomly around the tree and treated.

Treatments. Treatments consisted of light manipulation techniques viz. covering leaves behind fruit with a shade cloth, pruning shading leaves above the tagged shoot to improve light distribution and covering fruit with brown paper bags and carbohydrate allocation manipulations such as removing leaves from behind fruit on bearing shoots, scoring scaffold branches and hand thinning to one fruit per shoot. All treatments were carried out after the physiological fruit drop on 29 Nov. 2007 or on 3 Dec. 2008, respectively.

Measurements and data collection. Treated fruit were harvested on (14 May 2008) or (5 May 2009). In 2008, the sampled fruit were assessed for creasing severity, fruit rind colour, fruit diameter, rind thickness, albedo mineral concentration and in 2009 sampled fruit were only assessed for creasing severity and fruit rind colour. Creasing severity was evaluated on a score of 0 to 4 by dividing each fruit into four equivalent imaginary spheres and if no sphere was creased, it was designated as a zero until four if all spheres showed creasing. Creasing incidence as a percentage of fruit evaluated was also calculated. Fruit rind colour was determined based on the CRI fruit rind colour chart (Set No: 34)

for oranges (Appendix 1), with a range between one and eight, one being completely orange and eight being green. The fruit diameter of each fruit was measured using an electronic calliper (CD-6″ C, Mitutoyo Corp, Toyko, Japan). The albedo of the inside of the fruit was removed dried in an oven at 60^{O}C for 3 days and stored in vials for complete mineral analysis. Rind thickness was measured on the outside (sunny side) of treated fruit with an electronic calliper (CD-6″ C, Mitutoyo Corp, Toyko, Japan).

Statistical analysis. Analysis of variance was performed using the computer program SAS (Statistical Analysis System) Enterprise Guide 3. Duncan's multiple range test at P= 0.05 was used to test the treatment effects. All percentages were arcsine transformed to degrees asin√ before the statistical calculations were made.

Results

Creasing incidence and creasing severity on 'Washington' navel oranges in 2008 was 31.50% and 0.59 respectively and the severity of creasing was significantly increased by covering fruit with brown paper bags (Table 1). Fruit with their leaves covered with a shade cloth and fruit covered with brown paper bags had significantly greener fruit than the control fruit at harvest. Fruit with their leaves covered with a shade cloth, fruit from pruned trees and fruit covered with brown paper bags were significantly smaller than the control fruit at harvest. Fruit with their leaves covered with a shade cloth and fruit covered with brown paper bags had significantly thinner rinds than control fruit at harvest.

[Table 1]

Negative and weak correlations exist between rind thickness or fruit diameter and creasing incidence or creasing severity were observed in this study (Table 2). Very weak and non significant correlations between creasing incidence or creasing severity and macronutrient or micronutrient concentrations in the albedo tissue were also observed (Table 2).

[Table 2]

When comparing the inside (shady) with the outside (sunny) part of the fruit, the shady part of the fruit had a greater creasing severity than the sunny part of the fruit in all the treatments (Table 3).

[Table 3]

Nitrogen (N) and potassium (K) concentrations in the albedo tissue were significantly higher in fruit with their leaves covered with a shade cloth and fruit covered with brown paper bags (Table 4). Covering leaves behind fruit significantly increased the phosphorus (P) concentration in the albedo. Calcium (Ca) and magnesium (Mg) concentrations in the albedo were not significantly affected by any of the treatments.

[Table 4]

Covering fruit with brown paper bags significantly increased the manganese (Mn) concentrations in the albedo, but none of the other micronutrient concentrations in the albedo tissue were significantly affected by any of the treatments (Table 5).

[Table 5]

In 2009, a low incidence of creasing occurred compared to 2008 (31.5 %) on 'Washington' navel oranges in Citrusdal and none of the treatments resulted in significant effects on creasing incidence and creasing severity (Table 6). However, scoring scaffold branches resulted in significantly better fruit rind colour while hand thinning to one fruit resulted in significantly greener fruit compared to the control at harvest (Table 6).

[Table 6]

Discussion

In both years creasing incidence and creasing severity was not significantly affected by any of the treatments except for creasing severity in 2008 which was increased by covering fruit with brown

paper bags. Therefore, it is assumed that light could be involved in the development of creasing. There was also a tendency for the shady side of the fruit to have a significantly higher creasing severity compared to the sunny side of the fruit concurring with previous reports (Le Roux and Crous, 1938; Fourie and Joubert, 1957; Jones et al., 1967 and Holtzhausen, 1981). In the first year, covering leaves behind the fruit with a shade cloth and covering fruit with brown paper bags had a negative effect on fruit rind colour, however in the second year only hand thinning to one fruit per shoot had a negative effect on fruit rind colour, while scoring scaffold branches resulted in significantly better fruit rind colour at harvest

Fruit size and thickness were significantly reduced by covering the leaves with a shade cloth and the fruit with brown paper bags. Fruit from pruned trees were also significantly smaller than the control fruit at harvest. However, negative weak correlations between rind thickness or fruit diameter and creasing incidence or creasing severity were observed. Therefore, creasing incidence was not limited only to smaller fruit with thinner rinds which agrees with the findings of du Plessis and Maritz (2004) who could not establish a relationship between rind thickness and the occurrence of creasing in South Africa. However, in general creasing incidence is more pronounced on smaller fruit with thinner rinds than larger fruit with thicker rinds (du Plessis and Maritz, 2004; Jones et al., 1967; Le Roux and Crous, 1938).

The light manipulation techniques used on the leaves and fruit in this experiment significantly increased the N, P, K and Mn concentrations in the albedo tissue. However, the relationship between macro- and micro nutrients in the albedo tissue and creasing severity could not be established and was confirmed by very weak correlations between creasing incidence or creasing severity and mineral nutrient concentrations in the albedo tissue. Therefore, the reported negative correlation between Ca and creasing incidence and the positive correlations between K and creasing incidence (Storey et al., 2002) were not confirmed by this study.

In conclusion, covering fruit with brown paper bags increased creasing severity. Light manipulation techniques significantly increased N, K, P and Mn concentration in the albedo while carbohydrate manipulations did not influence mineral nutrients distribution in the albedo. Therefore, light influences creasing severity and most likely the distribution of mineral nutrients in the albedo tissue. The effect of carbohydrate manipulation techniques in relation to creasing incidence and albedo mineral concentration could not be established. It should be noted that in both years creasing incidence was low compared to commercial reports and it is possible that treatment effects will be different with higher creasing incidence. Therefore, additional research is required to further understand the effect of light manipulation techniques and the effect of carbohydrate manipulation techniques in relation to the incidence and severity of creasing. Furthermore, the results indicate that pruning to ensure light penetration in the tree may be important in reducing creasing incidence.

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Table 1. The effect of light and carbohydrates allocation manipulation techniques on the creasing incidence, creasing severity, fruit rind colour, fruit diameter and rind thickness of 'Washington' navel oranges in Citrusdal in 2008.

Treatment	**Creasing incidence**	**Creasing severity**	**Fruit rind colour**	**Fruit diameter**	**Rind thickness**
	---%---	*--0-4[x]--*	*--1-8[y]--*	*--mm--*	*--mm--*
1. Control	31.50	0.59 bc[z]	3.53 cd	65.65 a	4.96 a
2. Covering leaves with shade cloth	21.67	0.29 c	4.15 a	59.47 b	4.51 b
3. Pruning	35.13	0.54 bc	3.52 cd	61.49 b	5.12 a
4. Covering fruit with brown bags	35.38	1.12 a	3.94 ab	60.22 b	4.36 b
5. Removing leaves from behind fruit	19.25	0.30 c	3.77 bc	65.25 a	4.98 a
6. Scoring scaffold branches	36.75	0.47 bc	3.42 d	65.08 a	5.00 a
7. Hand thinning	38.00	0.68 b	3.54 cd	65.59 a	4.90 a
P-value	0.3998	0.0005	0.0001	0.0001	0.0005

[z]Means with the same letter are not significantly different at the 5% level (Duncan)
[y] 1-8 (1= orange, 8= green) CRI fruit rind colour chart (Set No: 34) for oranges
[x] 0-4 (0= 0%, 1= 25%, 2= 50%, 3= 75%, 4= 100%)

Table 2. Relationship between creasing incidence (%) or creasing severity and rind thickness, fruit diameter and macronutrient or micro nutrient concentrations in the albedo tissue of 'Washington' navel fruit exposed to different light and carbohydrate allocation manipulation techniques harvested in Citrusdal in 2008.

Rind thickness vs. creasing severity, creasing %			
		r	*P*-value
Rind thickness	Creasing %	-0.24	0.0853
Rind thickness	Creasing severity	-0.21	0.1341
Fruit diameter vs. creasing severity , creasing % and rind thickness			
Fruit diameter	Creasing %	-0.12	0.3945
Fruit diameter	Creasing severity	-0.14	0.3152
Fruit diameter	Rind thickness	0.33	0.0141
Creasing % vs. mineral nutrient in albedo			
Creasing %	N	-0.07	0.6059
Creasing %	P	-0.07	0.6267
Creasing%	K	0.00	0.9904
Creasing%	Ca	-0.22	0.1161
Creasing%	Mg	0.12	0.3879
Creasing %	Na	0.25	0.0708
Creasing%	Mn	-0.02	0.8878
Creasing %	Fe	0.03	0.8341
Creasing %	Cu	-0.11	0.4386
Creasing %	Zn	0.04	0.7928
Creasing %	B	0.17	0.2270
Creasing severity vs. mineral nutrients in albedo			
Creasing severity	N	0.01	0.9270
Creasing severity	P	-0.04	0.7557
Creasing severity	K	0.15	0.2674
Creasing severity	Ca	-0.26	0.0595
Creasing severity	Mg	-0.04	0.7974
Creasing severity	Na	0.19	0.1766
Creasing severity	Mn	0.01	0.9710
Creasing severity	Fe	0.04	0.7602
Creasing severity	Cu	-0.12	0.3823
Creasing severity	Zn	-0.06	0.6495
Creasing severity	B	0.16	0.2309

Table 3. Differences in creasing severity between the outside (sunny) and inside (shady) part of fruit exposed to different light and carbohydrate allocation manipulation techniques on 'Washington' navel trees harvested in Citrusdal in 2008.

Treatment	**Sun**	**Shade**	***P*-value**
	--------0-2--------		
1. Control	0.12	0.50	0.0001[z]
2. Covering leaves with shade cloth	0.02	0.27	0.0036[z]
3. Pruning	0.06	0.49	0.0001[z]
4. Covering fruit with brown bags	0.19	0.56	0.0001[z]
5. Removing leaves from behind fruit	0.02	0.28	0.0004[z]
6. Scoring scaffold branches	0.07	0.39	0.0001[z]
7. Hand thinning	0.10	0.57	0.0001[z]

[z]Means for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (t-test)

Table 4. Macronutrient concentrations in the albedo tissue of fruit sampled from 'Washington' navel trees exposed to different light and carbohydrate allocation manipulation techniques harvested in Citrusdal in 2008.

Treatment	**N**	**P**	**K**	**Ca**	**Mg**
	----------%----------				
1. Control	0.48 b[z]	0.030 bc	0.24 b	0.48	0.061
2. Covering leaves with shade cloth	0.56 a	0.035 a	0.34 a	0.48	0.068
3. Pruning	0.44 b	0.028 bc	0.23 b	0.48	0.069
4. Covering fruit with brown bags	0.55 a	0.031 ab	0.32 a	0.47	0.070
5. Removing leaves from behind fruit	0.45 b	0.030 bc	0.25 b	0.51	0.065
6. Scoring scaffold branches	0.46 b	0.029 bc	0.22 b	0.52	0.069
7. Hand thinning	0.46 b	0.026 c	0.25 b	0.45	0.060
P-value	0.0001	0.0072	0.0001	0.1870	0.0750

[z]Means in each column with the same letter are not significantly different at the 5% level (Duncan)

Table 5. Micronutrient concentrations in the albedo tissue of fruit sampled from 'Washington' navel oranges exposed to different light and carbohydrate manipulation techniques harvested in Citrusdal in 2008.

Treatment	Na	Mn	Fe	Cu	Zn	B
	----------*mg·kg^{-1}*----------					
1. Control	244.37	5.86 bc[z]	104.8	1.42	6.29	21.21
2. Covering leaves with shade cloth	255.91	6.94 ab	153.6	1.49	7.22	20.02
3. Pruning	257.21	4.79 c	33.5	1.19	6.02	19.99
4. Covering fruit with brown bags	250.79	8.43 a	292.2	1.60	6.25	21.74
5. Removing leaves from behind fruit	263.97	6.45 abc	138.4	1.43	6.92	21.37
6. Scoring scaffold branches	267.57	5.74 bc	98.7	1.21	6.11	20.05
7. Hand thinning	261.91	6.67 abc	196.2	1.43	5.87	19.50
P-value	0.3165	0.0146	0.0778	0.1519	0.1422	0.5569

[z]Means in each column with the same letter are not significantly different at the 5% level (Duncan)

Table 6. The effect of light and carbohydrates allocation manipulation techniques on the creasing incidence, creasing severity and fruit rind colour of 'Washington' navel oranges in Citrusdal in 2009.

Treatment	**Creasing incidence**	**Creasing severity**	**Fruit rind colour**
	---%---	*--0-4[x]--*	*--1-8[y]--*
1. Control	17.59	0.25	4.18 b[z]
2. Covering leaves with shade cloth	11.43	0.14	4.30 ab
3. Pruning	20.13	0.39	4.21 b
4. Covering fruit with brown bags	19.13	0.26	4.30 ab
5. Removing leaves from behind fruit	21.00	0.33	4.09 bc
6. Scoring scaffold branches	15.00	0.23	3.85 c
7. Hand thinning	13.29	0.16	4.52 a
P-value	0.8456	0.2618	0.0001

[z]Means with the same letter are not significantly different at the 5% level (Duncan)
[y] 1-8 (1= orange, 8= green) CRI fruit rind colour chart (Set No: 34) for oranges
[x] 0-4 (0= 0%, 1= 25%, 2= 50%, 3= 75%, 4= 100%)

7. EFFECT OF DIFFERENT ROOT BIOSTIMULANTS ON THE INCIDENCE AND SEVERITY OF CREASING

ABSTRACT. Creasing, also known as albedo breakdown is a physiological disorder that affects the albedo of citrus fruit. The physiological basis of creasing is still unresolved, but several hypotheses have been proposed. A lack of soil fertility was considered to be one of the important causes of creasing development. 'Washington' navel orange trees in Citrusdal were used to evaluate the effect of different root biostimulants such as humic acid, fulvic acid, coarse compost, and chicken manure on creasing incidence. Humic acid and fulvic acid were applied monthly from full bloom until a month before harvest in 2009. Coarse compost was applied at full bloom while a split application of chicken manure was done during the growing season on 23 Oct. 2008 and 11 Dec. 2008. Creasing incidence, creasing severity and fruit rind colour were not significantly affected by any of the treatments. Leaves from trees supplied with chicken manure had a significantly lower concentration of calcium at harvest. The pH, the phosphorus concentration, the cation exchange capacity and the base saturation of the soil were not affected by any of the treatments. In this study, the effect of different root biostimulants on the incidence and severity of creasing could not be established therefore it could not be recommended at this stage, however, it is possible that treatment effects could be observed over a longer period of time.

Introduction

Creasing is a pre-harvest physiological disorder of citrus, of which the physiological basis is unknown. The potential of the albedo cells to expand and accommodate cell enlargement after cell division has ceased has been identified as critical in creasing development (Holtzhausen, 1981; Storey and Treeby, 1994). Therefore the incidence and severity of creasing could be influenced by factors such as nutrition and soil condition and as early as 1938, a lack in soil fertility was considered to be an important factor in creasing development in South Africa (Le Roux and Crous, 1938). Jones et al.

(1967) observed that split applications of nitrogen (N) resulted in less creasing than did single applications of N in February northern hemisphere (NH). However, a high N treatments which are accompanied by an increase in yield, increases the incidence and severity of creasing (Embleton et al., 1973; Fourie and Joubert 1957; Jones et al., 1967). The application of phosphorus (P), especially in the absence of potassium (K), aggravates the incidence of creasing (Embleton et al., 1973; Fourie and Joubert, 1957; Jones et al., 1967) while the application of K fertilizers was reported to decrease creasing of 'Valencia' oranges (Jones et al., 1967).

Few studies have been done on the effect of soil condition in relation to creasing incidence, but it is recommended that soils with *Phytophtora* root rot problems and excessive nematode populations should be treated, while heavy and saline soils should be avoided. Nematode control results in more favourable assimilate partitioning and improved root health and activity (Miller and Hofman, 1988). Moreover, it is possible that improving the root activity is not only favourable for the uptake of nutrients and water but also for the synthesis of cytokinins, since root tips are a site for cytokinin synthesis (Salisbury and Ross, 1992; Van Staden and Cook, 1986). The production practices to improve soil health and root growth could to some extent improve cell division in the fruit and thereby have an influence on the development of creasing.

Generally, the use of different root biostimulants can change the chemical and physical properties of a soil over an extended period of time (Canali et al., 2004: Marinari et al., 2000) and could improve the tree performance (Ferguson et al., 2006; Webb et al., 1988). Canali et al. (2004) observed that six years of compost and chicken manure applications, soil fertility was improved and the soil properties slightly affected. The use of vermicompost or dairy manure over a period of 18 months improved the physical and biological properties of the soil (Marinari et al., 2000). On the contrary, Ferguson et. al. (2006) observed that chicken manure in combination with wood chips did not significantly affect soil fertility over a period of 18 months.

The aim of the study was to investigate the effect of different root biostimulants such as humic acid, fulvic acid, coarse compost, and chicken manure on creasing incidence. Humic acid is a dark brown solution and fulvic acid is a golden yellow solution and can be used as a soil application. These products have been marketed as biostimulants that can improve the properties of the soil and improve plant growth but remain to a large extent untested.

Material and methods

Plant material 'Washington' navel orange trees were used for this study in 2009 in a commercial orchard with a history of severe creasing incidence. This orchard is situated in Citrusdal (32°36'S, 19°1'E) in the Western Cape, South Africa. The tree spacing of this orchard was 6m between rows and 6m in rows and the row orientation was north to south. The experiment was laid out as a randomized complete block design with six treatments and ten single tree replicates.

Treatments. Treatments consisted of different root biostimulants such as humic acid (10 $L \cdot ha^{-1}$), fulvic acid, (20 $L \cdot ha^{-1}$), coarse compost (16680 $L \cdot ha^{-1}$) and chicken manure 2 x 2720 kg (72 $kgN \cdot ha^{-1}$). Treatment solutions of humic acid at 72 $mL \cdot tree^{-1}$ and fulvic acid at 36 $mL \cdot tree^{-1}$ were applied with 10 L of water per tree applied to the soil using a watering can along the drip line of the tree and wetting the whole area towards the tree trunk. These treatments were applied at full bloom on 6 Oct. 2008 and then repeated monthly during the growing season until a month before harvest on 2 Apr. 2009. Coarse compost at 60 $L \cdot tree^{-1}$ was placed as a mulch around the tree trunk at least up to the drip line of the tree at full bloom on 6 Oct. 2008. At two applications, 20 kg (0.52 kgN $tree^{-1}$) of chicken manure was placed around the tree trunk, up to the drip line of the tree on 23 Oct. 2008 and 11 Dec. 2008.

Leaf and soil analysis. At the time of application of the treatments on 6 Oct. 2008, the pH, phosphorus (P) content and CEC of the soil were determined. During the growing season on 20 Apr. 2008, 10 leaves were sampled from eight single tree replicates and a complete leaf mineral analysis was carried out. Soil sampling was also carried out during the growing season on 23 Apr. 2009 at two

depths, 0-30 cm and 30-60 cm. These samples were used for a pH, phosphorus (P) content and CEC analysis.

Measurements and data collection. An on-tree evaluation was conducted at commercial harvest on 5 May 2009. Fruit was only assessed for creasing severity and fruit rind colour. Creasing severity was evaluated on a score of 0 to 4; the orange was divided into four equivalent imaginary spheres. If no sphere was creased it was designated a zero, for a sphere that showed creasing the score was noted from 1 (one sphere) to 4 (all spheres of the fruit). Creasing incidence as a percentage of the fruit evaluated was also calculated. Fruit rind colour was determined based on the CRI fruit rind colour chart (Set No: 34) for oranges (Appendix 1), with a range between one and eight, one being completely orange and eight being green.

Statistical analysis. Analysis of variance was performed using the computer program SAS (Statistical Analysis System) Enterprise Guide 3. Duncan's multiple range test at P= 0.05 was used to test the treatment effects. All percentages were arcsine transformed to degrees asin√ before the statistical calculations were made.

Results

Low creasing incidence (9.4%) and creasing severity (0.11) occurred on 'Washington' navel orange fruit in Citrusdal in 2009 (Table 1). Creasing incidence, creasing severity and fruit rind colour were not significantly affected by any of the treatments (Table 1).

[Table 1]

The application of chicken manure resulted in significantly lower concentrations of calcium (Ca) in the leaves when compared to the other treatments and the untreated control (Table 2). None of the other leaf macronutrients (Table 2) or any of the leaf micronutrients (Table 3) were significantly affected by any of the treatments.

[Table 2 and 3]

Similarly the pH, the phosphorus (P) concentration, the cation exchange capacity (CEC) (Table 4) and the base saturation of the soil (Table 5) were not significantly affected by any of the root biostimulant applications on 'Washington' navel orange trees in Citrusdal.

[Table 4 and 5]

Discussion

None of the treatments resulted in a significant reduction in creasing incidence and creasing severity in this study. Fruit rind colour was also not affected by any of the treatments. However, the use of root biostimulants may have the potential to improve the fruit rind quality given that tree growth can be improved by the application of humates (Webb et al., 1988) and chicken manure in combination with wood chips (Ferguson et al., 2006). Ferguson et al. (2006) also reported that the application of a combination of chicken manure and wood chips increased the concentrations of P, K and magnesium (Mg) in the leaves. However, in our studies trees applied with chicken manure had significantly lower concentrations of Ca in the leaves compared to the other treatments and the untreated control.

In our studies, the pH, the phosphorus (P) concentration, the cation exchange capacity (CEC) and the base saturation of the soil were not affected by any of the treatments. In general, root biostimulants change the chemical properties and physical properties of a soil (Marinari et al., 2000). The use of organic material such as vermicompost, dairy manure over a period of 18 months has been reported to improve the physical and biological properties of the soil (Canali et al., 2004; Marinari et al., 2000). On the contrary, Ferguson et. al. (2006) observed that chicken manure applied alone or in combination with wood chips did not significantly affect soil fertility over a period of 18 months and it was suggested that organic materials should affect soil fertility over longer term experiments as observed by Canali et al. (2004). It should be noted that creasing incidence was very low, varies between seasons (Fourie and Joubert, 1957; Holtzhausen, 1981) and that the study was conducted in

one year only. Therefore, it is possible that the treatment effects would be more pronounced over a long period of time and with higher creasing incidence.

In conclusion, the different root biostimulants did not reduce the incidence and severity of creasing and could not be recommended at this stage, however, it is possible that treatment effects could be observed if the experiment was extended over a longer period of time. Therefore, additional research on the effect of root biostimulants on root activity and the incidence and severity of creasing should be evaluated over a longer period of time.

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Table 1. The effect of different root biostimulants on creasing incidence, creasing severity and fruit rind colour 'Washington' navel oranges in Citrusdal in 2009.

Treatment	**Creasing severity**	**Creasing incidence**	**Fruit rind colour**
	--0-4[x]--	*---%---*	*--1-8[y]--*
1. Control	0.11	9.44	3.97
2. Humic acid	0.15	10.62	3.93
3. Fulvic acid	0.12	9.09	3.98
4. Chicken manure	0.13	9.44	4.27
5. Compost	0.23	12.78	3.94
P-value	0.1515	0.8925	0.2016

Means were separated at the 5% significance level (Duncan)
[y] 1-8 (1= orange, 8= green) CRI fruit rind colour chart (Set No: 34) for oranges
[x] 0-4 (0= 0%, 1= 25%, 2= 50%, 3= 75%, 4= 100%)

Table 2. Leaf macronutrient concentrations of 'Washington' navel orange trees treated with different root biostimulants harvested in Citrusdal in 2009.

Treatment	N	P	K	Ca	Mg
	----------%----------				
1. Control	2.06	0.088	0.738	3.21 a[z]	0.263
2. Humic acid	2.21	0.093	0.730	3.12 a	0.348
3. Fulvic acid	2.18	0.093	0.778	3.18 a	0.330
4. Chicken manure	2.15	0.098	0.873	2.79 b	0.278
5. Compost	2.08	0.090	0.783	3.22 a	0.300
P-value	0.5052	0.3848	0.1553	0.0229	0.1323

[z]Means with the same letter are not significantly different at the 5% level (Duncan)

Table 3. Leaf micronutrient concentrations of 'Washington'navel orange trees treated with different root biostimulants harvested in Citrusdal in 2009.

Treatment	Na	Mn	Fe	Cu	Zn	B
	----------*mg·kg^{-1}*----------					
1. Control	402.75	30.00	79.50	4.25	35.75	151.08
2. Humic acid	296.50	21.50	60.25	3.75	31.50	109.25
3. Fulvic acid	375.25	25.00	71.50	3.74	32.75	125.50
4. Chicken manure	355.25	24.75	71.00	3.75	32.73	117.75
5. Compost	360.50	27.00	128.00	3.00	36.00	134.25
P-value	0.2305	0.0828	0.5258	0.6605	0.3812	0.1394

[z]Means with the same letter are not significantly different at the 5% level (Duncan)

Table 4. The pH, phosphorus (P) content and cation exchange capacity (CEC) of soil sampled from 'Washington' navel orange trees treated with different root biostimulants in Citrusdal in 2009.

Treatment	**pH**	**P**	**K**	**Na**	**Ca**	**Mg**
		--mg·kg^{-1}--	*----------cmol(+)·kg^{-1}----------*			
1 Control	5.52	22.60	0.172	0.152	2.04	0.67
2. Humic acid	5.46	30.80	0.178	0.106	1.94	0.63
3. Fulvic acid	5.52	43.20	0.160	0.096	2.09	0.63
4. Chicken manure	5.58	35.40	0.214	0.112	2.24	0.73
5. Compost	5.36	39.20	0.156	0.090	1.72	0.59
P-value	0.7201	0.2457	0.3721	0.4071	0.7172	0.7132

Means were separated at the 5% significance level (Duncan)

Table 5. Base saturation of soil sampled from 'Washington' navel orange trees treated with different root biostimulants in Citrusdal in 2009.

Treatment	**Na**	**K**	**Ca**	**Mg**
	----------%----------			
1. Control	4.86	5.24	60.04	20.50
2. Humic acid	3.69	5.81	59.41	19.81
3. Fulvic acid	3.15	5.25	61.57	19.93
4. Chicken manure	3.14	6.08	63.48	20.42
5. Compost	3.29	5.42	59.33	19.57
P-value	0.5229	0.9044	0.8986	0.9884

Means were separated at the 5% significance level (Duncan)

8. OVERALL DISCUSSION AND CONCLUSION

The objectives of the study were to determine if the bearing position of fruit in a tree, light manipulation techniques, carbohydrates manipulation techniques and the albedo mineral nutrient concentrations throughout the season and at harvest contribute to creasing development. Furthermore, the most effective application timing of gibberellic acid (GA_3), with the least negative effect on fruit rind colour development and as well as the effectiveness of cytokinins, other products and different root biostimulants were tested for their efficacy in reduction of creasing incidence.

Our results showed that the position of fruit in the tree plays a significant role in creasing development. The fruit from the inside of the tree canopy had a significantly higher creasing incidence compared to the outside fruit. In addition, creasing incidence was higher on the south side of the tree than on the north side of the tree. Therefore, with large differences in creasing incidence amongst different sub-sectors in the tree, selective harvesting may be an option to reduce the proportion of creased fruit in the packhouse.

In addition, it was also observed that light levels in the tree canopy could play an important role in creasing development due to the tendency of the inside (shady) part of outside fruit to have a higher creasing severity than the outside (sunny) part of the fruit. Additionaly, creasing severity was significantly increased when fruit were covered with brown paper bags. Hence, pruning to ensure light penetration in the whole tree canopy could be important in reducing creasing incidence. In addition, in the orchard it is important for the growers to look on the inside of outside fruit when evaluating creasing incidence.

Generally, the concentration of mineral nutrients in the albedo were influenced by the fruit position within the the tree and as well as light levels in the tree canopy. The light manipulation techniques such as covering leaves behind fruit with a shade cloth or covering fruit with brown paper bags

significantly increased N, P, K and Mn concentrations in the albedo tissue at harvest. Significant differences in the albedo mineral nutrients of fruit sampled from the different sub-sectors were observed, but no inverse or similar trends as for creasing incidence or creasing severity were observed. Therefore, it could be concluded from this study that albedo mineral concentrations at harvest did not contribute to creasing development. This was confirmed by the non significant correlations between creasing incidence or creasing severity and the albedo mineral concentrations at harvest. However, it is possible that albedo mineral nutrient concentrations earlier in the season play a role in creasing development, as significant correlations between creasing severity and Cu, K, and Mn concentrations in the albedo during stage II of fruit growth were observed. Therefore, additional research on the relationship between creasing severity and the albedo mineral nutrients earlier in the season would be recommended.

Creasing incidence and albedo mineral nutrients were not affected by any of the carbohydrate manipulation techniques used in this study. This could be because creasing incidence was low, compared to a high creasing season and it is possible that treatment effects will be evident with higher creasing incidence. Therefore, additional research is required to further understand the effect of carbohydrate manipulation techniques in relation to creasing incidence and albedo mineral nutrients.

Application of GA_3 significantly reduced the incidence and severity of creasing, irrespective of the application timing. The application of GA_3 resulted in a delay in fruit rind colour development with the weakest fruit rind colour development resulting from the mid January GA_3 applications. In order to achieve significant reductions in creasing incidence, GA_3 foliar applications from mid November until mid January is recommended. GA_3 spray applications later than mid January should be avoided, due to a stronger inhibition of the fruit rind colour development.

Localised fruit application of CPPU [N-(2-chloro-4-pyridyl)-N-phenylurea], MaxCel (6-Benzyladenine) and a mixture of CPPU and calcium after physiological fruit drop reduced the

incidence and severity of creasing, although creasing incidence was not significantly different from the control. It was observed that the mixture of CPPU and calcium was not as effective as the single application of CPPU. Cytokinins applied alone may have a potential to reduce creasing incidence, therefore further studies on the methods of application and uptake efficiency of cytokinins are recommended, because in citrus the uptake and translocation of applied cytokinins are not very effective. Application of Messenger® and AVG (aminoethoxyvinylglycine) did not reduce creasing incidence and cannot be recommended.

In our study, the effect of different root biostimulants on the incidence and severity of creasing could not be established therefore cannot be recommended. However, it should be noted that creasing incidence was very low and that the study was conducted in one year only. It is possible that the treatment effects could be more pronounced if the study was done over a longer period of time and with a higher creasing incidence.

Appendix 1. CRI fruit rind colour rating chart for oranges